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GROWTH AND MINERALS: ZINC

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INTRODUCTION

Zinc (Zn) is well known to be essential for somatic growth of children. Zinc has a close relationship with the endocrine system; it sustains normal growth, secondary sex characteristics, reproductive function and thyroid function. Therefore, Zn deficiency causes not only growth retardation, but also delayed sexual maturation, hypogonadism, and thyroid dysfunction. In this paper, the effects of Zn on childhood growth are presented.

Highlights In This Issue

E-Abstracts (Abstracts On-line)

From The Editor's Desk

Those of you who have followed this column may be aware of the trials and tribulations of the recent past, as *GGH* faced an uncertain future. But, we are back on track and are delighted to advise you that *GGH* will forge ahead.

This issue brings to an end the era of the long-term sponsorship of *GGH* by Genentech, Inc. They generously supported this educational vehicle since its inception in 1985. On behalf of our readers and Editorial Board I extend our thanks for what they did for the journal. Their support allowed *GGH* to become established and develop into a highly sought-after journal. *Growth, Genetics & Hormones* is read by most pediatric endocrinologists worldwide and other specialists interested in the field of growth.

I am happy to report that we will enjoy an unrestricted educational grant from our new sponsor, INSMED (Glen Allen, Virginia). Thanks to them, we will continue publishing *GGH* as we have done for two decades and you will continue to receive *GGH* on a complementary basis.

While searching for the means to continue *GGH*, I was very motivated by countless colleagues who wrote of the high value they placed on *GGH*; many were willing to pay for a subscription to the journal if needed. I thank all of you who encouraged me, and in this way helped with the task of eliciting funding to serve the educational goals of our colleagues.

The Editorial Board has pledged their time and effort to review the latest advances in the field and grace us with their insightful comments. I am looking forward to a new era of *GGH* and to continue bringing you the most updated reviews and lead articles of interest to the readership. We will continue to enhance the impact of *GGH* and strive to ensure that readers continue to enjoy and treasure it.

Please keep me posted of your needs and recommendations for continuous enhancements. There are multiple journals and other means to stay informed, but none like this journal. Join me in extending your thanks and appreciation to our past sponsor and a heartfelt welcome and thanks to our new sponsor, INSMED, for making this possible.

Respectfully,
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Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

THE ROLE OF ZN ON THE HOMEOSTATIC MECHANISMS THAT AFFECT GROWTH AND GROWTH HORMONE

Zinc ion (Zn^{2+}) is present in high concentrations in the somatotrophs in the anterior pituitary of rats, chiefly localized in the growth hormone (GH) secretory granules, and to a smaller extent in the Golgi apparatus. Particle induced X-ray emission (PIXE) measurements reveal that the content of Zn in the anterior pituitary is significantly different between male and female rats (100.5 ± 7.0 vs 74.2 ± 3.6 [SD] ng/mg dry weight,[1] respectively). On the other hand, in human subjects, the anterior pituitary of women contains more Zn than that of men, but the concentration of Zn in young males is higher than that of young females.[2] However, the reason for the sex difference of Zn content of the pituitary gland is not clear.

Growth hormone is synthesized and secreted into storage granules before its release from the anterior pituitary. Zinc induces GH dimerization; two Zn ions associate per dimer of GH in a cooperative fashion. The Zn^{2+}-GH dimer is more stable than monomeric GH and the formation of the dimeric complex is considered to be important for storage of GH in secretory granules.[3] However, the function of Zn in the release of GH from the somatotrophs is not known.

The mechanism by which Zn deficiency causes growth disturbance is considered controversial. Zinc is required for the activity of more than 200 enzymes (Zn metalloenzymes) in which Zn is located at the active site, including DNA polymerase, RNA polymerase, and thymidine kinase. In general, Zn serves catalytic, co-catalytic, and/or structural functions in metalloenzymes containing this ion. Because these enzymes are important for nucleic acid and protein synthesis and cell division, Zn is considered to be essential for growth. Furthermore, several hundred Zn-containing nucleoproteins are probably involved in the gene expression of various proteins.[4] The molecular mechanisms by which Zn controls the expression of the insulin-like growth factor (IGF)-I and the growth hormone receptor/growth hormone binding protein (GHR/GHBP) genes remain unsettled.[5]

Zn seems to play a role in the intracellular transduction pathways of several hormones and might activate protein kinase C which could play a role in the transduction of the GH signal.[6] Zn is an essential component of the "Zn-finger" structures which function as the DNA-binding domains of transcription factors. Zinc-finger is a structure in which an atom of Zn is tetrahedrically coordinated to spatially conserved cysteines and histidines; the Zn atom is absolutely required for binding to DNA.[7] The presence of Zn in these proteins is essential for site-specific binding to DNA and gene expression. Zn serves as a strut that stabilizes folding of the domain into a finger loop, which is then capable of site-specific binding to double-stranded DNA.

The Zn-finger loop proteins provide one of the fundamental mechanisms for regulating gene expression of many proteins. It is estimated that there may be approximately 200 to 300 Zn-finger nucleoproteins involved in gene expression. Whether or not Zn deficiency affects these nucleoproteins and gene expression remains to be demonstrated.[4] Nuclear receptors of several hormones–including steroid hormones and thyroid hormones–contain Zn-finger structures. Therefore, Zn deficiency might cause alterations of these hormonal actions through the dysfunction of Zn-finger proteins.

The presence of a large amount of Zn in bone tissue suggests that this ion also plays an important role in the development of the skeletal system.[8] Zinc has a stimulatory effect on bone formation and mineralization,[9] whereas retardation of bone growth is a common finding in various conditions associated with Zn deficiency. Zn is required for the action of alkaline phosphatase (ALP) activity, this enzyme is mainly produced by osteoblasts whose major function is to provide calcium deposition in bone diaphysis. Zinc increases the half-life of ALP activity in human osteoblast-like cells.[10]

The administration of both Zn or vitamin D_3 produced a significant increase in bone ALP activity and DNA content, and the effect of vitamin D_3 was synergistically enhanced by the simultaneous treatment with Zn.[11] The receptors for 1,25-dihydroxyvitamin D_3 were shown to have two Zn-finger structures at the site of interaction with DNA.[12] One possible function of Zn is to potentiate the interaction of the 1,25-dihydroxyvitamin D_3-receptor complex with DNA.

Zinc directly activates aminoacyl-tRNA synthetase in osteoblastic cells, and it stimulates cellular protein synthesis. Moreover, Zn inhibits osteoclastic bone resorption by suppressing osteoclast-like cell formation from marrow cells. Zinc may act on the process of bone-resorbing factors induced by protein kinase C activation; these are involved in Ca^{2+} signaling in osteoclastic cells.[9]

OPTIMAL AND SUBOPTIMAL ZN NUTRITURE

It has been estimated that the body of the infant newborn contains approximately 60 mg of Zn based on a concentration of 20 µg/g of tissue.[13] During growth and maturation, Zn concentration of the human body increases to approximately 30 µg/g. The adult total body Zn content ranges from about 1.5 g in women to 2.5 g in men.[14] Thus Zn nutrient intake is essential and is particularly important in rapidly growing children, adolescents, as well as pregnant and lactating women.

RDA of Zn in the United States[15]

Age	Zn mg/day
normal infants from birth to 12 months of age	5
children 1 to 10 years of age	10
males older than 11 years of age	15
females older than 11 years of age	12
pregnant women	15
lactating women	
first 6 months after delivery	19
second 6 months after delivery	16

The recommended dietary allowances (RDA) of Zn in the United States are listed (Table). The RDA is neither the minimal requirement nor necessarily the optimal level of intake. Rather, the RDA is a safe and adequate level, incorporating margins of safety intended to be sufficiently generous to encompass the presumed variability in requirements among individuals, reflecting the state of knowledge concerning a nutrient, its bioavailability, and variations among the population.[15]

Zinc nutriture has been a subject of worldwide concern as a public health problem. The mean and median intakes of Zn reported in 171 studies summarized by the International Atomic Energy Agency ranged from 4.2 to 19 mg/day; the 10th, 50th, and 90th percentiles of intake were 7, 10, and 14 mg/day, respectively.[16] Zinc intake varies with the mode and type of feeding. Zinc intake of breast-fed infants ranged from 1.9 mg/day at 1 month of age to 2.7 mg/day at 6 months, and those of bottle-fed infants were 3.6 and 4.6 mg/day at 1 and 6 months, respectively.[17] However, Zn in human milk is absorbed more efficiently than that in bovine milk. Absorption of Zn was 41 ± 9 % (SD) from human milk, 28 ± 15% from cow's milk, 31 ± 7% from humanized cow's milk formula, 22 ± 11% from cereal-cow's milk formula, and 14 ± 4% from soy formula.[18]

Total dietary Zn intake is greatly influenced by food choices. Animal products provide abundant amount of Zn and cereals supply the primary plant source. However Zn intake is correlated with protein intake and is markedly influenced by the protein source. Diets consisting primarily of eggs, milk, poultry, and fish have lower Zn:protein ratios than those composed of shellfish, beef, and other red meats. Similar variations occur in vegetarian diets. Diets with rich Zn:protein ratios are provided by liberal quantities of legumes, whole grains, nuts, and cheese, whereas those with low ratios are contained primarily fruits and vegetables.[19]

Zinc absorption is a function of the solubility of Zn compounds at the absorption site and the body status or need. Zinc bioavailability is defined as the fraction of Zn intake that is retained and used for normal physiologic functions. Meats, liver, eggs, and seafood are considered good bioavailable sources of Zn because of the relative absence of compounds that inhibit its absorption, as well as the presence of certain amino acids that improve Zn solubility.[19] For example, the absolute amount of Zn absorbed was about 80% higher when a high meat diet (280 g meat/day) was consumed than with a low meat diet (42 g meat/day).[20] On the other hand, whole-grain cereal products and plant proteins, such as soy protein, contain Zn in a less available form. The phytic acid content of plant foods accounts for, at least in part, to the lower availability of Zn from these foods. Dietary fiber is considered to have little or no effect on Zn availability.[19]

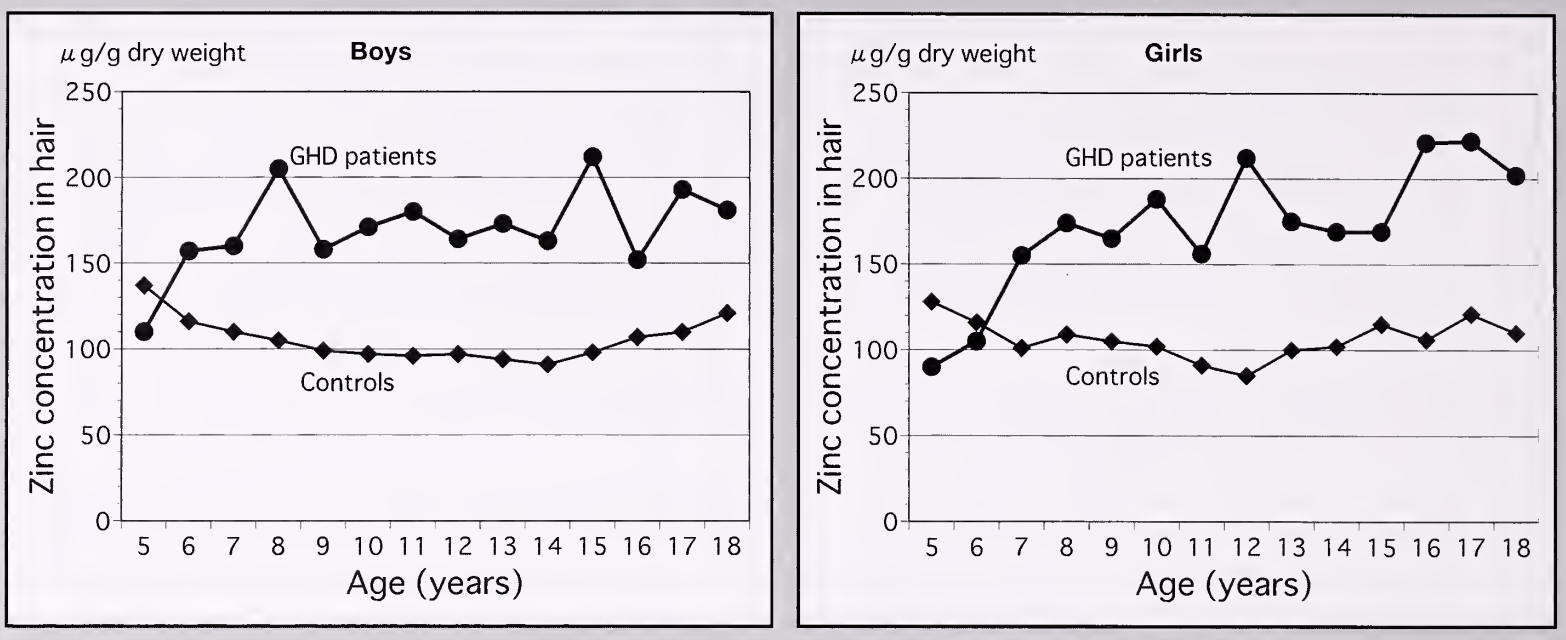
EFFECTS OF ZN DEFICIENCY AND MARGINAL ZN DEFICIENCY ON GROWTH AND GROWTH HORMONE

It is well known that Zn deficiency causes growth retardation in children and adolescents. Patients with growth retardation caused by Zn deficiency were first described by Prasad et al[21] in 1963. These patients presented with short stature and hypogonadism; their diets were lacking in protein and were rich in phytate and fiber. They were shown to have Zn deficiency by decreased Zn concentrations in plasma, erythrocytes, and hair. Furthermore, ^{65}Zn studies revealed that plasma Zn turnover was greater, the 24-hour exchangeable Zn pool was smaller, and the excretion of ^{65}Zn in stool and urine was less in the growth-retarded subjects than in the controls.[21] The growth velocity was increased and was greater in those who received supplemental Zn than those receiving only an adequate animal protein diet.[4] Since then, many cases of marginal or moderate growth impairment in children with Zn deficiency as a consequence of an inadequate Zn nutriture have been reported from various regions of the world.[22,23]

Zinc deficiency is also known to affect GH metabolism and the concentration of GH also influences or is associated with changes in the concentrations of Zn in blood, urine, and other tissues.[8] In patients with GH deficiency (GHD) the mean plasma Zn concentration was within normal limits before treatment, but was significantly reduced after 4 to12 months of GH administration. The urinary excretion of Zn was significantly higher than that of controls before treatment and was decreased after GH therapy.[24] The average Zn concentration in hair of GHD patients given GH therapy was about 1.7 times higher than that of the controls (Figure), and the hair Zn concentrations of newly diagnosed GHD patients significantly increased after GH administration.[25]

On the other hand, in patients with acromegaly there was a negative correlation between plasma Zn and serum GH levels, and a positive correlation between urinary Zn excretion and serum GH levels. After hypophysectomy, Zn was observed to increase in plasma and decrease in urine.[24] These findings may reflect a negative Zn balance

Mean zinc concentrations in hair of GHD patients and the controls 5-18 years of age. [25]



and chronic mild Zn deficiency in some GHD patients on long-term GH therapy and in untreated patients with acromegaly. The data suggest that an increased Zn requirement exists during catch-up growth or overgrowth accelerated by GH, and that GH might promote intestinal absorption of Zn and/or promote Zn uptake of hair root cells. It may also be speculated that Zn may be a limiting factor in growth-regulating mechanisms by modulating both GH release and GH action.[8]

Zinc deficiency may adversely affect GH production and/or secretion.[26] IGF-I synthesis may also be impaired by Zn deficiency since exogenous GH fails to raise IGF-I levels in Zn-deficient rats.[27] Low IGF-I levels in Zn-deprived rats were closely associated with a decreased hepatic IGF-I gene expression and with a diminution of liver GH receptors and circulating GHBP. The decreased hepatic GH receptors and/or GHBP concentrations might be responsible for the decline of circulating IGF-I in Zn-deficient animals.[28]

The incorporation of labeled thymidine into DNA is also impaired by Zn deficiency. This effect has been detected within a few days of the institution of a Zn-deficient diet in experimental animals, suggesting that DNA biosynthesis[4] is compromised due to an adverse effect of Zn restriction on the activity of deoxythymidine kinase.[29]

There have been a few reports concerning the relationship between Zn deficiency and GH secretory insufficiency in humans. We described a 13-year-old Japanese patient with short stature who had partial GH deficiency due to chronic mild Zn deficiency.[26] This patient's diet was low in animal protein and consisted primarily of rice and vegetables (he disliked meats, fish, eggs, and dairy products) and plasma Zn level and GH responses to pharmacological stimulation tests were low. After 3 months of oral Zn supplementation, the patient's growth velocity improved without GH replacement therapy, and the plasma Zn levels and GH responses to stimulation tests normalized.

On the other hand, Siklar et al[30] investigated the Zn nutriture of prepubertal GHD patients given GH treatment in Turkey. They measured erythrocyte Zn levels and reported that about one-half of them were Zn deficient. Growth velocity during GH treatment was higher in children with normal erythrocyte Zn levels than those with low erythrocyte Zn concentrations. They also showed that oral Zn supplementation improved the growth velocity of GHD children with Zn deficiency, but not of those without Zn deficiency. These data indicate that Zn status should be evaluated before GH provocative tests and during GH treatment.

MATERNAL ZN NUTRITURE AND PREGNANCY OUTCOME

It has been well known that Zn deficiency during pregnancy may be associated with increased maternal morbidity, prolonged gestation, inefficient labor, atonic uterine bleeding, and increased risks to the fetus.[4] Maternal Zn deficiency may also cause intrauterine growth retardation (IUGR) and low-birth-weight (LBW) infants.[31-33] The Zn levels of polymorphonuclear and mononuclear white cells in postpartum women at 24 to 48 hours after delivery were lower in women giving birth to small-for-gestational-age (SGA) infants than those giving birth to appropriate-for-gestational-age (AGA) infants, irrespective of smoking habits.[31] A significant correlation existed between maternal plasma Zn concentrations measured at mid-pregnancy and an infant's birth weight. The maternal weight at 3 months of gestation and plasma Zn concentrations in the second trimester formed the best predictor model of birth weight.[32] It was also reported that the prevalence of LBW infants was significantly higher (8 times) among women with serum Zn concentrations in the lowest quartile in early pregnancy, independent of other

risk factors.[33] However, there have been other studies that showed no association between maternal Zn nutriture and pregnancy outcome.[34,35] It is also known that plasma Zn concentrations are not reliable indicators of the Zn status and are not useful in estimating marginal Zn deficits.[36]

The effects of Zn supplementation on pregnancy outcome are not clear.[37-40] The incidence of LBW is very high in many developing countries where Zn deficiencies are prevalent. For example, an estimated 40% to 50% of all live births in Bangladesh were classified as LBW, 70% to 80% of which were the result of IUGR.[40] Effective interventions aimed at preventing LBW are particularly important to reduce childhood malnutrition and improve infant health. In developing countries maternal Zn supplementation has been suggested as one possible nutritional intervention during pregnancy to improve pregnancy outcomes.[41] Studies of Zn supplementation during pregnancy have been positive and resulted in reduced incidence of IUGR.[38,39] In a randomized, double-blind, placebo-controlled trial in 580 African-American women, Zn supplementation (25 mg/day) during pregnancy was associated with an increase in birth weight (+126 g) as compared with infants of women who received placebo.[39]

However, the results of Zn-supplementation trials in pregnant women aimed to improve pregnancy outcome are not consistent.[40] A double-blind, prospective study carried out in the United Kingdom found no differences in gestational age, birth weight, neonatal abnormalities, and complications of labor and delivery between mothers given a Zn supplement and those given a placebo.[37] It is now speculated that Zn supplementation during pregnancy might be beneficial only in populations that are Zn deficient and at high risk for poor fetal growth.[40]

PREVALENCE OF ZN DEFICITS IN HEALTH AND DISEASE STATES

The population groups at risk of Zn deficiency are those who consume low Zn-quality diets. Such diets are rich in phytate and usually contain other ligands that prevent the intestinal absorption of Zn.[42] On a global scale, protein energy malnutrition is the most common cause of poor growth and short stature, and it appears that Zn deficiency is also prevalent in such populations.[4] Stunted growth linked to Zn deficiency was found throughout childhood, and depending on the country, 5% to 30% of children were suffering from moderate Zn deficiency, resulting in for small-for-age height.[43] However, in recent experimental studies in rats, suboptimal nutrition restricted growth primarily when energy was not ingested in sufficient quantities, whereas suboptimal intake of Zn with an appropriate intake of calories did not stunt growth.[44]

Several studies indicated that marginal Zn deficiency might also be prevalent in infants and children in developed countries. Michaelsen et al[45] investigated Zn intake and status in healthy term infants from birth to 12 months of age in Denmark, and found suboptimal Zn status in many subjects during late infancy. They also reported that serum Zn levels at 9 months of age were positively correlated with growth velocity during the period from 6 to 9 months of age. We studied Zn status in short Japanese children with normal GH secretion using the body Zn clearance test to detect marginal Zn deficiency, and found that about 60% of the short children had such a problem. The reason for the high incidence of marginal Zn deficiency in Japanese short children may be due to the recent dietary preference for precooked meals, snacks and convenience foods.[46]

Disorders of the gastrointestinal tract are frequently complicated with Zn deficiency. Breakdown of the integrity of the gastrointestinal tract reduces the normal absorption of dietary Zn and disrupts the enteropancreatic circulation of the ion.[19] There is evidence that patients with Crohn's disease, sprue, or short bowel syndrome may develop Zn deficiency. Several investigators have reported low serum Zn concentrations present in 30% to 70% of patients with Crohn's disease,[47-49] and it is not unusual to find depressed urinary Zn excretion.[50] It has been reported that about 20% to 30% of children with Crohn's disease have severe linear growth retardation, mainly due to malabsorption and malnutrition.[51] On the other hand, it has been reported that about 30% to 70% of children with Crohn's disease have reduced serum Zn levels. Brignola et al[52] evaluated the effect of oral Zn supplementation on serum Zn levels in patients with Crohn's disease with hypozincemia and concluded that administration of very high doses of Zn (200 mg/day $ZnSO_4$) for 3 months increased serum Zn levels, but that moderate doses (60 mg/day) did not. We studied Zn status in 30 patients with chronic inflammatory bowel disease (CIBD) and found that 11 subjects had hypozincemia. In addition, those with moderate and severe clinical disease activity had a decreased rise of serum Zn concentration after oral Zn administration. Urinary excretion of Zn after oral load was also remarkably low in all CIBD patients. The abnormalities of Zn metabolism were more frequent among the CIBD patients with growth abnormalities, although they were also found in normal height patients.[51]

GROWTH ENHANCEMENT CAPABILITIES OF ZN IN "HEALTHY" INFANTS AND CHILDREN

There have been several reports indicating positive effects of oral Zn supplementation on growth of SGA and/or LBW infants fed artificial formulas.[45,53,54] In a longitudinal, double-blind, randomized clinical trial in preterm infants in Spain, those fed standard milk formula supplemented with Zn for 6 months had greater linear growth velocity corrected for postnatal age than those without Zn supplementation. Zinc supplementation significantly increased serum and erythrocyte Zn levels and serum ALP activity,[53] but no differences were induced in serum IGF-I and IGFBP-3.

IGF-I and IGFBP-3 are of course essential for linear growth in children from childhood to adolescence, but might not be as important for neonates and young infants. There was also a positive effect of Zn supplementation on linear growth in SGA infants fed artificial formula, but not in those fed exclusively breast-milk.[54] This may be attributed to the lower bioavailability of Zn contained in formula compared to the Zn in human milk, placing formula fed infants at a higher risk of Zn deficiency. Therefore, the effect of Zn supplementation on artificially fed infants would be more evident.[53] Mild Zn deficiency in SGA and LBW infants, especially those fed artificial formula, could be a public health problem even in developed countries.

There are several studies that assessed the effects of Zn supplementation on children's growth.[36,46,55,56] Nakamura et al[36] conducted an age-matched control study and showed that oral Zn supplementation was effective in improving the growth rate of short children with marginal Zn deficiency. They also reported that oral Zn supplementation induced increases of serum IGF-I, osteocalcin, and ALP activity.

The effects of oral Zn supplementation were evaluated in short Japanese children with normal GH secretion assessed for Zn status with a body Zn clearance test.[46] The results indicated that oral Zn supplementation was effective on height gain in short boys with marginal Zn deficiency, but not in girls. There was a significant correlation between the body Zn clearance values and the increase in the growth velocity after oral Zn supplementation in boys, indicating that the degree of Zn deficiency was important. Although the reasons for the difference in the effects of oral Zn supplementation on growth velocity between both sexes are not clear, other studies showed similar differences,[55] oral Zn supplementation improved growth velocity in boys with idiopathic short stature, but had no effect in girls. On the other hand, a relatively large scale randomized, double-blind, placebo-controlled study showed no positive effect of Zn supplementation on height gain of preschool children.[56]

The results of many other studies are also inconsistent. Brown et al[57] completed a meta-analysis of randomized controlled intervention trials to assess the effect of Zn supplementation on the physical growth of prepubertal children. After evaluating 33 reports, they found that 26 studies showed positive effects of Zn supplementation on children's linear growth and 7 studies did not. They concluded that interventions to improve children's Zn nutriture should be considered in populations at risk of Zn deficiency, especially where there are high rates of underweight or stunted growth.

ASSESSMENT OF ZN DEFICIENCY AND MARGINAL ZN DEFICIENCY

Unfortunately there is no simple, accurate way, to determine the Zn status of individuals, and this is the major factor that handicaps the interpretation of the data of most studies and of individual patients. There have been various kinds of laboratory biomarkers proposed to detect definite and/or marginal Zn deficiency. However, these measurements do not accurately reflect nutritionally available Zn pool sizes.[19]

Although plasma/serum Zn concentration has been widely used to assess the nutritional status, Zn levels may respond to metabolic conditions unrelated to Zn status and are insensitive to changes in dietary Zn.[58] The insensitivity of plasma Zn to reductions in dietary Zn intake reflects the tremendous capacity of the organism to conserve tissue Zn by reductions in Zn excretion and/or reductions in the rate of growth. A reduction in plasma Zn concentration does not occur until the capacity to reestablish homeostasis by reducing excretion and/or growth has been exceeded. Plasma Zn represents about 2% of a labile, or nutritionally available, total-body Zn pool that exchanges with isotopic Zn tracers in 24 hours.[58] Because plasma Zn is the source of this ion for all tissues, plasma concentrations are maintained longer than other components of the body Zn pool.[19]

Plasma Zn kinetics or turnover tends to increase with Zn depletion. Thus, the rate of Zn turnover in the plasma compartment or in the total labile pool of the body might indicate the Zn status of an individual. Miller et al[59] estimated the size of the combined pools of Zn with which plasma Zn exchanged using isotopic Zn. The exchangeable Zn pool size was determined from the amount of isotope introduced into the plasma and the coefficient of the simple exponential decay function fitting enrichment data between day 3 and 9 after isotope administration. They reported that the exchangeable Zn pool size correlated with habitual dietary Zn intake. This excellent assay to detect marginal Zn deficiency may be of little practical use in the clinical situation because of the necessity for isotope administration.

Nakamura et al[36] recommended a body Zn clearance test which needs no isotope. This is a kind of a Zn kinetic study; serum Zn levels are measured just before and at 30, 60, 90, 120 minutes after intravenous administration of Zn, the serum Zn decay curve is obtained, and the biological half-life and elimination constant of serum Zn are calculated. The resultant "body Zn clearance" value becomes a sensitive indicator of marginal Zn deficiency.

Other static measurements of Zn status hold little promise. Erythrocyte Zn is mildly affected by Zn deficiency and may not be a sensitive index. The response of leukocyte Zn to changes in Zn status is not consistent among laboratories, and the assay is laborious.[19] Hair Zn levels may be depressed in mild Zn deficiency. However, it is affected by the rate of hair growth and shows seasonal variations.[60] Urinary excretion rates of Zn are diminished

in severe deficiency states, but this measurement is not sensitive and is confounded by many clinical disorders that increase urinary Zn losses.[19]

SUMMARY AND SPECULATION

Zinc, although present in minute quantities in humans, is an essential nutrient and plays an important role as a component of many enzyme systems regulating cell growth, including DNA and protein synthesis, energy metabolism, regulation of gene transcription, hormone levels, and growth factor metabolism.

Nutritional Zn deficiency is still a worldwide public health problem. In developing countries, protein energy malnutrition is the most common cause of poor growth and short stature of children, and Zn deficiency is prevalent in such populations. Zn deficiency in pregnant women is also a serious problem, since it might cause IUGR and LBW infants. Since the incidence of LBW is very high in many developing countries, Zn supplementation in pregnant women should be considered extensively in such regions.

Marginal to moderate Zn deficiency is not uncommon even in developed countries. Zn deficiency should be considered as one of etiologic factors in some children with unexplained short stature. Oral Zn supplementation may be considered as the growth-promoting therapy for children with short stature once marginal Zn deficiency is established. However, the interrelationships among Zn, growth, gonadal function, and GH-IGF-I axis appear to be complex and deserve further investigation.

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ABSTRACTS FROM THE LITERATURE

Summary Highlights: ESPE and LWPES Joint Meeting

Summarized here are some highlights of the joint meeting of ESPE and LWPES in Lyon, France, September, 2005.

CONGENITAL HYPERINSULINISM

The molecular basis of congenital hyperinsulinism in infancy (CHI) was reviewed by de Lonlay et al (S7-30) from Paris. CHI is characterized by severe dysregulation of insulin secretion that causes profound hypoglycemia. It is associated with either focal or diffuse pathology of the endocrine pancreas. These pancreatic anatomical forms of pathology require major differences in the treatment of CHI.

Mutations in genes encoding the beta-cell sulfonylurea receptor (SUR1) and the inward-rectifying potassium-channel (Kir6.2) have been identified in CHI. These genes encode subunits of KATP channels which couple glucose metabolism to insulin release. Hypoglycemia is related to homozygosity of a paternally inherited mutation of one of these genes that results in diffuse hyperplastic pancreatic pathology. The CHI is more complex in the *diffuse* form which present as a heterogeneous disorder involving several genes and various inheritances. Forms occurring in the CHI, resistant to medical treatment, are mostly due to mutations of the KAPT channel with a recessive inheritance and often require total pancreatectomy. Forms occurring after the first month of life are mostly sensitive to medical treatment and may be related to *de novo* or dominantly inherited mutations. Nonetheless about half of the patients in the later group do not carry these mutations.

Focal lesions in CHI represent areas of adenomatosis related to the loss of the maternal allele in the 11p15 region. It is mostly sporadic. This somatic molecular event disrupts the balanced expression of imprinted genes involved in the control of cell growth and lead to pancreatic tumor development. This clinical form of CHI is potentially curable by limited pancreatic resection. Until recently rather invasive techniques, with direct pancreatic catheterization using transhepatic portal venous sampling, and arterial calcium-stimulated venous sampling were used to aid the surgeon with the localization and the extent of the pancreatectomy. In localizing pancreatic focal lesions Otonkoski (S7-31) from Helsinki successfully used the PET technique to detect neuroendocrine tumors using (18F)-DOPA uptake by the hyperplatic islet cells. Very promising results were also obtained by several other centers, including Blankenstein et al (P3-1260) from Berlin and Stanley et al (S7-32 and P3-1262) from Philadelphia. All patients with positive PET technique localization of a focal lesion had the tumor removed while preserving the healthy portion of the pancreas and the hypoglycemia ceased. Stanley et al described their experience in 217 cases with neonatal hypoglycemia over 6 years. They confirmed the accuracy of the PET scan technique performed before surgery and recommended that candidates for surgery be referred to specialized centers.

GROWTH HORMONE TREATMENT

Simon et al (OR3-75) from Paris reported on the early recombinant human growth hormone (rhGH) treatment started one year after initiation of glucocorticoid therapy of children with juvenile rheumatoid arthritis. This randomized-controlled study was carried out over 3 years in prepubertal children receiving prednisone (0.6 ± 0.4 mg/kg/day) and rhGH (0.46 mg/kg/week). Growth was maintained at a normal rate for chronological age and lean body mass was improved. However, there was no significant effect on fat mass or bone mineralization. Although increased insulin resistance was expected with rhGH therapy, glucose intolerance was mild and transient, occurring only in pubertal patients. It was concluded that rhGH treatment was safe and prevented growth retardation in these patients. However, higher steroid doses may limit the beneficial effects of rhGH. Thus rhGH treatment may prove to be more significant when given early as prevention of growth retardation. Further studies that follow patients until final height is achieved and focus on muscle mass and long-term function are in progress.

Mauras et al (P1-149) from Jacksonville reported on the limited efficacy of rhGH treatment during the so-called transition period in a well defined cohort: children with GH deficiency (GHD). Subjects had been treated early and reached normal final height, metabolic control, muscle strength, and bone mineral density (BMD). The authors delved with the question of the timing of rhGH treatment: namely the continuation or the temporary discontinuation throughout late adolescence to adulthood, until the persistence of GHD could be re evaluated. A phase III double-blind, randomized 2-year trial was performed. Subjects were classified in 3 groups: persistently GHD randomized to either continued rhGH treatment or to placebo injections, and GH sufficient on retesting considered controls and given no treatment. After 2 years metabolic measures, cardiac function, BMD, and quality of life were comparable in all 3 groups. It was concluded that GHD adolescents in good metabolic control at time of epiphyseal fusion may safely discontinue rhGH therapy for at least 2 years.

If such an attitude is chosen, a careful follow-up is needed to determine if and when rhGH is warranted. Such an approach may help manage the so-called transition period before adulthood in previously well treated GHD patients. (Refer to abstract on page 14.)

IGF-I

Camacho-Hubner, Savage, and Underwood (S10-40) from London and Chapel Hill updated their experience with rh insulin-like growth factor (IGF)-I alone or combined with rhIGF binding protein (BP)-3 in patients with GH insensivity (GHI) due to GH receptor defects, to growth attenuating antibodies to GH, or to extreme insulin resistance due to genetic defects. The doses of rhIGF-I ranged from 60 to 120 mg/kg/day. Height improved by 1.2 to 1.5 SD over 2 years of therapy; the best responses occurred when the treatment was started at a young age. However long-term responses varied among treated children and were not as well sustained as the responses elicited with rhGH therapy in GHD children. Adverse events were: coarsening of facial features, hypoglycemia in younger children, lymphoid hyperplasia, and pseudotumor cerebri. The combined rhIGF-I and IGFBP-3 (0.5 to 2 mg/kg/day) given as a single injection, resulted in a prolonged half-life of IGF-I, allowing once daily injection with appropriate tolerance and good results. The only treatment available for patients with severe genetic insulin resistance and genetic IGF defects is rhIGF-I. A multicenter open labelled phase III study is in progress in children with GHI.

GENETICS OF GH RECEPTOR

A recent issue of interest focuses on genetic factors possibly influencing the growth response to rhGH therapy. A provocative, well documented study of GH gene polymorphism and variations in growth in GH treated children by Bougneres et al (S3-20) from Paris investigated the potential role of the 2 forms of the GH receptor (GHR), the full length (fl) or the exon-3 deleted (d3) receptor, on the response to rhGH treatment. In transfection experiments a 30% increase in the transduction in GH signalling had been demonstrated in d3 homo or heterodimers of the GHR. This suggested that there could be a potential genetic factor influencing the response to rhGH. An advantage for the d3-allele carriers was shown in children with small for gestational age or idiopathic short stature (ISS) who showed 1.7 to 2 times greater growth acceleration as compared with those who did not have this GHR. These results were confirmed in a cohort with complete GHD treated for 3 years by Thomas-Teinturier (P1-152) from Paris.

Two other studies were at variance with these data. Blum et al (OR3-71) from Lilly's Genesis Program studied a large cohort of GHD children treated with rhGH (0.2 $\pm$ 0.06 mg/kg/week). This group evaluated the first year of rhGH treatment response according to the exon-3 genotype and reported a small but consistent (although not significant) increase in growth parameters in the d3 groups. Ito et al (P1-150) from Japan evaluated Japanese children with partial GHD and could not demonstrate a significantly increased growth during the first rhGH treatment year in patients with 3d allele.

However, the initial finding by Bougneres remains an important and provocative paper that will generate future studies to better understand the large individual differences in growth response to rhGH. The role of exon-3 genotype must be confirmed with strong methodological approaches selecting groups according to etiology of short stature, rhGH dosage, duration of treatment, and ethnicity before it can be applied as a genomic tool for therapy.

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CONGENITAL ADRENAL HYPERPLASIA

The presence of testicular adrenal rest tumors in congenital adrenal hyperplasia (CAH) patients is known to cause Leydig cell failure and impaired spermatogenesis. These rest tumors are often unresponsive to intensified corticoid therapy. Bachelot et al (OR 14-142) from Paris reported treatment of 3 adult patients with mitotane, an adrenolytic agent, for 2 to 3 years and obtained a reduction in the testicular rest tumor volume with an improved sperm count. This may represent a potent tool to improve fertility of some poorly controlled CAH patients.

OVARIAN FAILURE

The causes of premature ovarian failure (POF) are rarely identified in adults. However Conway (S9-38) from London approached this issue with data related to optimization of the substitutive estrogenic therapy in adolescents. Age at onset of this treatment was critical for adult carotid intima media thickness, predictive of vascular complications, and was inversely correlated with the estrogen dosage. Appropriate uterine thickness for future pregnancy was obtained if treatment was not delayed. Finally, better results in assisted conception with donated oocytes were also obtained in women with early ovarian failure who received treatement before the age of 14. These data may also be relevant to patients with gonadal dysgenesis and those with ovarian failure secondary to cancer therapy in pediatric practice.

Raphaël Rappaport, MD

Measured versus Reported Parental Height

Cizmecioglu and colleagues interviewed 200 parents (100 males, 100 females), mean age 37.8 years and ascertained their reported height. Their actual height was then measured by a single observer using a Harpenden stadiometer. On average, males overestimated their height, while females reported their height relatively accurately. However, there was a wide spread of discrepancies for both sexes. Overall there was a small positive correlation between age and the difference between reported and measured height. Of interest, subjects who had been measured previously were less accurate at reporting their height than those who guessed their height. The mean difference in reported versus measured height was 1.09 cm for men (range −3.3 to 5.2) and −0.09 for females (range −6.2 to 6.4). The authors pointed out that there was considerable individual variation among both sexes in over or under estimating their exact height and state that their data reinforces the need for accurate height measurement and recording of both mother and father at the earliest possible opportunity.

Cizmecioglu F, Doherty A, Paterson WF, Young D, Donaldson MD. Measured versus reported parental height. Arch Dis Child. 2005;90:941–942.

Editor's Comment: *This is a very short paper which represents some interesting and very important information. It is a relatively common practice in pediatric endocrine clinics to calculate the mid-parental height as a target height for the child being evaluated. Clearly it is important that this target height is calculated correctly. It is not uncommon for parents to state that they are unaware of their precise height or to report their height with obvious discrepancy from observation. In addition it is not uncommon for children to come the clinic with either one or more non-biological parents, or for information regarding the "no longer present" parent's height to be estimated with little precision. The recommendations of the authors of this study should be taken seriously: parental height should be measured at the earliest possible time and become part of the child's permanent medical record. Such information could be exceedingly helpful in guiding the evaluation and treatment of children with growth failure at a later date. At the very least, pediatricians and pediatric endocrinologists should be encouraged to actually measure parents who accompany their child for evaluation of growth failure.*

William L. Clarke, MD

Apnea in Prader-Willi Syndrome Patients on Growth Hormone Therapy

Case reports of sudden fatalities, primarily respiratory, in children with Prader-Willi Syndrome (PWS) receiving growth hormone (GH) therapy caused alarm and prompted a voluntary label change to include a new warning. Benefits of GH treatment in these patients include improved linear growth, increased muscle mass and amelioration of hypotonia, and decreased total body fat. Sleep-disordered breathing is common in PWS, both obstructive (from pharyngeal narrowing, respiratory muscle hypotonia, and later compounded by obesity) and central (hypothalamic dysfunction with abnormal arousal and response to hypercarbia which can be further blunted by obesity).

Miller and colleagues performed a prospective study of the respiratory effects of 6 weeks of GH treatment in 25 patients with genetically confirmed PWS. All patients underwent standard overnight polysomnography (PS) at baseline (either GH-naïve or voluntarily withdrawn from GH treatment for 3 months) and after 6 weeks of GH (0.24 mg/kg/wk for children and 0.0006 mg/kg/day for adults,

based on ideal body weight); two of the patients were also retested after 6 months. Subjects ranged in age from 5 months to 39 years. All had sleep-disordered breathing during the baseline PS, with both obstructive and central apneic events. After 6 weeks of treatment, 19 of the patients (76%) had improvement of the apnea/hypoxia index (AHI); the frequency of central events decreased by a median of 1.7 events/hr, while the frequency of obstructive events did not change significantly. However, 6 patients (24%) had worsening of obstructive sleep apnea/hypopnea, related to upper respiratory tract infections (URIs) and tonsillar hypertrophy. Two of these patients had high insulin-like growth factor (IGF)-I levels for bone age (z scores of +1 and +2; the others had IGF-I z scores of 0). After GH dose reduction and normalization of IGF-I level, one patient had an improved AHI on repeat PS while the other had increased AHI and a URI at the time of the repeat study. Body-mass index was not related to PS results.

The authors concluded that PS should be performed in all PWS patients at baseline, after 6 weeks of treatment with GH, and with otorhinolaryngolic evaluation whenever symptoms of sleep apnea or snoring develop. Adenotonsillectomy and titrating GH dose to achieve an IGF-I z score of 0 were also recommended as needed. Finally, they supported the warning of GH manufacturers contraindicating GH use in PWS patients with CRI or lung infections.

Miller J, Silverstein J, Shuster J, Driscoll DJ, Wagner M. Short-term effects of growth hormone on sleep abnormalities in Prader-Willi Syndrome. J Clin Endocrinol Metab. 2006;91:413–417.

First Editor's Comment: *I applaud the authors for performing a prospective study to directly address the question of GH effects on respiratory function in PWS patients, and I agree with the proposed pathophysiologic mechanisms. However, the finding of sudden death in individuals with hypothalamic dysfunction and the recurrent theme of exacerbation by intercurrent infections make me wonder about central adrenal insufficiency, which was not mentioned. Indeed, a PubMed search of adrenal insufficiency and PWS produced only one paper.*[1] *In this retrospective series report of 8 children and 2 adults with unexpected death or critical illness, 3 of the children had below-average sized adrenal glands on autopsy; childhood illnesses in general under the age of 2 years were associated with high fever and rapid demise or near-demise. Increased mortality among individuals with GH deficiency (GHD) despite GH treatment has been attributed to under-diagnosed and under-treated central adrenal insufficiency, and recent papers highlighted the increased risk for central adrenal insufficiency even in patients with idiopathic GHD or familial isolated GHD.*[2,3] *Thus, in addition to the recommendations by Miller et al, I would encourage monitoring of adrenal function in PWS patients.*

Adda Grimberg, MD

Second Editor's Comment: *Excellent points made by the authors of the paper and the editorial comment of Dr. Grimberg. I urge caution and continuous monitoring of PWS patients throughout their life, not just after initiating GH therapy, and particularly when ill.*

Fima Lifshitz, MD

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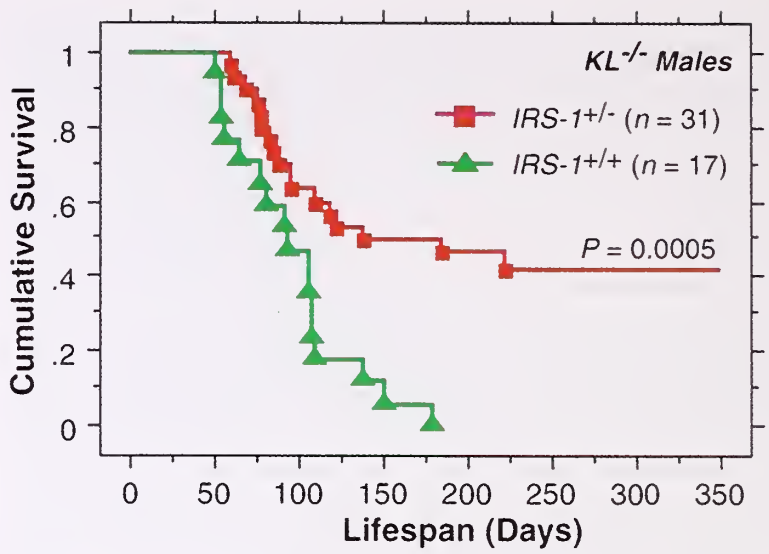
Suppression of Aging

A spontaneous homozygous loss-of-function mutation in *KLOTHO* (*KL*) gene (OMIM 604824, chromosome 13q12) was initially described in a strain of mice with accelerated aging and premature death.[1] Its human homolog was later identified. *KL* encodes a transmembrane protein expressed in renal distal convoluted tubules and neural choroid plexus. Kurosu et al developed 2 strains of transgenic mice that **overexpressed** *Kl* under the control of the promoter of human elongation factor 1α. Both male and female animals overexpressing *Kl* lived 20% to 30% longer than did wild-type (WT) control mice. They did so without restricting caloric intake or impeding somatic growth; however, fecundity was reduced in like-breeding pairs. Mice overexpressing *Kl* were euglycemic, but males had higher serum insulin concentrations than did WT controls, and both genders had attenuated hypoglycemic responses to exogenous insulin and/or insulin-like growth factor (IGF)-I. The serum concentration of the extracellular domain of Klotho was twice as high in transgenic as in WT mice. Intraperitoneal administration of Klotho protein increased blood glucose concentrations and depressed the hypoglycemic effect of co-injected insulin. *In vitro* in cultured cells, Klotho peptide did not inhibit binding of insulin or IGF-I to their specific receptors, but specifically suppressed autophosphorylation of these receptors and impaired insulin-stimulated glucose uptake. Furthermore, Klotho down-regulated intracellular signaling transmitted through insulin receptor substrate (IRS)-1 and -2 and phosphoinositide 3-kinase p85. In $Kl^{-/-}$ mice who die prematurely, life could be substantially prolonged and signs of aging halted (ie, arteriosclerosis, renal calcification, testicular atrophy) by decreasing a generation of IRS-1. The authors concluded that Klotho was a secreted protein (ie, a hormone) that extended life

and suppressed aging by antagonizing the cellular effects of insulin and IGF-I.

Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. Science. 2005;309:1829–1833.

Editor's Comment: *Klotho may be the long sought after elixir from the "fountain of youth." KLOTHO is named after the mythological Greek Fate who spun the "thread of life." By alternative RNA splicing, KL generates 2 transcripts: a 1012 amino acid protein with extracellular, transmembrane, and intracellular domains and a 549 amino acid peptide, the amino terminal sequence of the extracellular domain that is secreted and is the predominant form produced. In man, single-nucleotide polymorphisms in KL have been associated with altered life span and risk for atherosclerosis and osteoporosis.[2] That increased generation of Klotho extended life span without impairing growth emphasizes the distinctive difference between the effects of this gene and that related to caloric deprivation, another experimental mechanism to prolong life. Although both processes act by impeding insulin and IGF-I action, Klotho apparently enhances their production but antagonizes their function, while caloric deprivation depresses their production and impairs growth and fertility. These studies reinforce the concept that decreased secretion of growth hormone, insulin, and IGF-I extends life and suppresses aging,[3] a concept that is the opposite of that voiced by many lay "anti-aging authorities." Although excess Klotho decreased fecundity between like-breeding pairs of mice, the effect of this protein on the fertility of a mouse with a high level of Klotho when mated with a WT animal remains to be explored. Conceptually, there appears to be a "trade-off" between life span and reproduction. It will be of great interest to measure serum concentrations of Kotho at various stages of life and in various hormonal and metabolic disorders, particularly those involving energy utilization, as well as to determine its physiologic (and potentially therapeutic and anti-aging) effects in humans of all ages.*



Rescue of aging-like phenotypes in KL-/- mice by genetic intervention in insulin and IGF-1 signaling.
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Allen W. Root, MD

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Motivations for GH/GnRHa Treatment and Psychosocial Functioning

Visser-van Balen et al reported (in the first paper) on the psychological consequences of combined growth hormone (GH)/gonadotropin-releasing hormone agonist (GnGHa) treatment in a multicenter, randomized-controlled study conducted in early pubertal youths (ages 11 to 13 years; Tanner breast stage 2 or 3 for girls, Tanner genital stage 2 or 3 for boys) with a diagnosis of either idiopathic short stature (ISS; 17 girls, 9 boys) or born small for gestational age (SGA; 8 girls, 4 boys). The authors explained the unusual predominance of girls as reflective of the combination of SS and relatively early puberty is more common in girls that boys. Participants had a height SDS below –2, or between –1 and –2 with a predicted adult height SDS below –2. In the second paper, the authors examined patients' and parents' motivations in choosing to participate in this study.

Adolescents in the treatment group were administered GH (4 IU [1.33mg]/m^2 BSA, SQ, daily) and GnRHa (3.75 mg, IM depot, every 4 weeks). At baseline, 1, 2, and 3 years after beginning treatment, adolescents and their parents (mostly mothers) in both groups completed questionnaires to assess the psychosocial functioning of the adolescents by completing a standardized assessment evaluating adolescents' health-related development, current height-related stressors, and parental concerns about their child's future behavioral and emotional functioning; perceived current and expected adult height; global intelligence; perceived competence, psychological distress, and personality characteristics.

At baseline, a minority of parents (28%) reported their child experienced teasing or juvenilization by peers; however, a higher proportion (44.5%) anticipated their child would face challenges in the labor market as an adult (39% of boys, 48% of girls) and 39% expected their child to have lower prospects of finding a spouse (77% of boys, 17% of girls, $p<0.01$). Parent reports of behavioral and emotional functioning suggested a statistically significant excess of problems. In contrast, adolescents'

self-reports of emotional distress and self-concept did not systematically differ from normative values. Differences in psychosocial variables at baseline were not detected between the treatment and control groups, ISS and SGA subgroups, or children whose parents reported stature-related psychosocial stressors. With regard to motivation to participate, patients were categorized into 4 subgroups based on the presences of height-related psychosocial stressors, parental worries about their child's current behavior and about future prospects, and patients' self-reported problems in psychosocial functioning.

During treatment, parent reports of current stigmatization and worries over future challenges did not change, and did not differ between the treatment and control groups. The same was not true for perceptions of the child's behavioral and emotional functioning. In contrast, self-perceived scholastic and athletic competence in the treatment group significantly decreased over time (ie, became more negative), while that of the adolescents in the control group increased (moderate effect size). Trait anxiety decreased for adolescents in the control group, but remained at approximately the same level for adolescents in the treatment group. The authors noted that, despite these statistically significant effects, there was considerable overlap of scores between the 2 groups and one apparent outlier in the treatment group.

As noted above, parents perceived an excess of psychological adjustment problems in their children, however this difference was not matched by the children's self-reports. As such, the investigators concluded that it is primarily the parents' perceptions of problems (current or anticipated) that drive the process in search of a medical intervention. The adolescents wanted to gain height, but their underlying motivation remains unclear.

Visser-van Balen H, Geenen R, Moerbeek M, J et al. Psychosocial functioning of adolescents with idiopathic short stature or persistent short stature born small for gestational age during three years of combined growth hormone and gonadotropin-releasing hormone agonist treatment. Horm Res. 2005;64:77–87.

Visser-van Balen H, Geenen R, Kamp GA, Huisman J, Wit JM, Sinnema G. Motives for choosing growth-enhancing hormone treatment in adolescents with idiopathic short stature: a questionnaire and structured interview study. BMC Pediatr. 2005;5:15.

Editor's Comment: *My initial reading of this study left me somewhat confused: why would researchers look for effects of combined GH/GnRHa treatment on psychological outcomes when the long-term benefits of GnRHa on adult height had not yet been realized? In fact, the addition of GnRHa could have slowed growth. The answer to this puzzle is that this study was not about psychological effects of changes in height, but rather was examining the influence of arrested pubertal development on adolescents' psychosocial adaptation. An implicit assumption justifying GnRHa as an adjunct to GH treatment is that the benefits of taller adult height outweigh the potential psychosocial liabilities of delayed or arrested pubertal development. The findings of a more negative self-concept in the treatment group give reason for pause. There are many reasons for viewing these findings as tentative, not the least of which is the high rate of missing data by the third year of treatment, confounding interpretation of the findings.*

It was not so long ago that delayed puberty (in males, at least) was considered a significant threat to the individual's psychosocial development.[1,2] Perhaps the time has come to consider a head-to-head comparison of the short- and long-term psychological benefits of on-time puberty versus taller adult stature.

Few studies directly examine parents' motivations in seeking care for their child,[3,4] and no studies of the children themselves. This study begins the process of filling an important gap in knowledge. It has long been known that there is limited concordance in the reports of parents and their children when a description of the child's psychosocial adaptation is in question,[5] a clear limitation in employing "parent-proxy only" assessments. Another methodological cautionary note derives from the likelihood of overestimating the incidence of emotional/behavioral problems when comparing clinical samples to population norms. The authors correctly pointed out that norms for a commonly used behavior problem checklist, the Child Behavior Checklist, are biased towards mental health and not representative of the general population.[6]

This study documented that future (even more than current) worries about the short child, are on the minds of parents when they seek treatment for their child. To the extent that this finding is replicated in independent and larger studies, it suggests that parents' decisions may hinge predominantly upon the negative stereotypes of foreclosed life options for adults with short stature. The empirical basis for these stereotypes are shaky.[7,8] Accordingly, it is the clinician's responsibility to check for, and to correct these faulty assumptions when present, lest they engender self-fulfilling prophecies.

David E. Sandberg, PhD

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Efficacy of Growth Hormone During Transition from Adolescence to Adulthood in Patients with Growth Hormone Deficiency

Mauras and colleagues conducted a multicenter, double-blind, placebo-controlled 2-year follow-up study of 58 subjects (mean age 15.8 ± 1.8 years; 33 males) who were treated for GH-deficiency as children and who, upon retesting at near adult height, were still GH-deficient (GHD). The study consisted of 3 phases: a basal phase, a washout phase, and an assessment phase. Twenty-five subjects were enrolled in the GH group (15 males, 10 females), 15 in the placebo group (9 males, 6 females), and 18 in the GH-sufficient control group (8 males; 7 females) of which 3 were excluded from analysis because they had evidence of multiple anterior pituitary hormone deficiencies. Forty-two subjects completed the study period that included baseline assessment and follow-up assessments at 2, 4, 8, 12, 16, 20, and 24 months: 21 patients in the GH group, 11 in the placebo group, and 10 in the control group (assessed only at 12 and 24 months). The primary objective of the study was to establish the efficacy of GH treatment with regards to body composition and bone mineral density changes, as well as the safety of a transition dose (20 µg/kg/d) of GH as replacement therapy in subjects with GHD during the transition from adolescence to adulthood. Secondary objectives included exploring the effects of GH treatment on plasma lipids, insulin-like growth factor (IGF)-I concentration, carbohydrate metabolism, cardiac function, exercise tolerance, and quality of life (QoL).

The results, in general, failed to reveal a significantly beneficial effect of GH on measures of either body composition or bone mass over the 2-year study compared with the placebo group. There were also no measurable improvements in functional measures of muscle strength. Cardiovascular assessment revealed normal cardiac function and exercise tolerance in the study subjects at baseline and throughout the study. The lipid profile did not change during GH therapy, and measures of carbohydrate metabolism showed only mild increases in measures of insulin resistance. QoL measures were unchanged during the 24-month trial. The authors concluded that GHD adolescents who are in good metabolic status at the time of discontinuation of GH treatment may be able to discontinue GH for at least 2 years without any deleterious effects, and that replacement treatment in adulthood needs to be individualized.

Mauras N, Pescovitz OH, Allada V, Messig M, Wajnrajch MP, Lippe B for the Transition Study Group. Limited efficacy of growth hormone (GH) during transition of GH-deficient patients from adolescence to adulthood: A phase III multicenter, double-blind, randomized two-year trial. J Clin Endocrinol Metab. 2005;90:3946–3955.

First Editor's Comment: *The investigators provided several plausible explanations for the finding that treatment of GHD adolescents in transition to adulthood did not elicit metabolic or QoL benefits, including the younger age of these research participants than those in studies showing benefits, the brief length of time off of GH, a possibly over-liberal threshold for diagnosing persistent GHD (<5 µg/liter), and sample attrition.*

It is noteworthy that the QoL scores of the GHD participants were indistinguishable from those of the general population while on GH and prior to the washout phase of the study. Unfortunately, one can not surmise what the level of functioning was before initiating treatment years earlier. Without such baseline data, it would be erroneous to conclude that GH treatment in childhood and adolescence had any effects on QoL.

Finally, if the results of this well-designed study can be replicated, then this would come as good news to patients, families, and clinicians. No one, least of all the adolescent patient, looks forward to continuing daily injections beyond the period of active linear growth. Most GHD patients will end this initial phase at some point during adolescence, a phase of development notoriously difficult from the perspective of adherence to medical regimens.[1] *Knowing that no physical or psychological harm will come to patients by introducing a hiatus in treatment for at least 2 years provides the opportunity to re-educate the now increasingly mature patient about the changing hormonal requirements to support optimal health (physical and QoL).*

David E. Sandberg, PhD

Second Editor's Comment: *This work was presented at the ESPE – LWPES Joint Meeting and reviewed on page 8 of this issue of GGH.*

Fima Lifshitz, MD

Reference

1. La Greca AM, Bearman KJ. In: Roberts MC, ed. Handbook of Pediatric Psychology. New York: Guilford Press; 2003:119–140.

Pituitary GH-secretory Cells

Bonnefont and colleagues answered a long-standing question: if growth hormone (GH)-secreting cells are heterogeneously distributed and scattered throughout the anterior pituitary, as shown by histology, how do they physiologically mount GH pulsatile release that is frequently a thousand-fold in magnitude, especially since

their GH pulses are much smaller when studied *in vitro*?

Using GH-GFP transgenic mice and custom-made computer software, these investigators were able to identify and localize the 3-D position of the labeled somatotrophs within the pituitary gland. Examination of fixed pituitaries from adult male mice revealed a connected 3-D, multi-cellular system comprised of numerous intercrossing strands of single GH cells with larger cell clusters at the intersections. This GH multi-cellular assembly withstood dispersion by a high-pressure *in vivo* perfusion procedure, and was shown to be linked by focal adherens junctions containing β-catenin.

The system was shown to be both functional and plastic. Comparing the volume-to-surface ratios of the GH cell clusters within the lateral and median pituitary zones, the ratios were similar in prepubertal animals. However, GH cell clusters increased in the lateral zones from puberty to adulthood, and then returned to prepubertal geometries in the oldest mice. Cell clustering was prevented by prepubertal castration of male mice, without a significant change in GH cell density in the lateral zones; organizational geometry was the important factor for the pubertal increase in growth. Multi-cellular calcium recordings of GH-EGFP cells in acute pituitary slices were measured as a marker of cell-cell connectivity in hormone release. No large-scale cell connectivity was observed during spontaneous electrical activity. This increased in the lateral pituitary zones following GH-releasing hormone (GHRH) stimulation, leading to temporally precise, synchronized, recurrent calcium spikes that correlated with the frequency of small GH pulses reported in other studies; enzymatic dispersion of the GH cells prevented GHRH-stimulated calcium spike synchronization. GHRH also increased calcium spiking in the median pituitary zone by changing the cell connectivity into small islets of more highly functionally connected GH cells at some points in the system interspersed with functionally less connected GH cells.

The authors concluded that, "GH cells function as a geometry-driven network of cells, connected to each other by adherens junctions." It logically follows that disruption of network architecture constitutes a novel mechanism for impaired GH release in pathological conditions, an issue the authors are pursuing in follow-up experiments.

Bonnefont X, Lacampagne A, Sanchez-Hormigo A, et al. Revealing the large-scale network organization of growth hormone-secreting cells. Proc Natl Acad Sci. 2005;102:16880–16885.

Editor's Comment: *A 3-D approach to functional analysis of the GH cell network provided novel and interesting insights into its physiology that were heretofore unobtainable. Because it is noninvasive and provides sensitive, real-time data of cellular and molecular events within their biological context,[1] in vivo bioluminescent imaging has recently emerged as a powerful new approach to elucidate physiologic and pathophysiologic mechanisms. It can be used grossly, such as monitoring rejection of transplanted tissues[2,3] or growth of cancer metastases.[4] It can also be used to study protein-protein interactions,[5] transcription,[6] and gene silencing.[7] Bioluminescent or fluorescent imaging holds great promise as a means of drug testing, both for therapeutic efficacy[8] and potential effects on normal tissues,[9] as well as in vivo evaluation of gene therapy strategies.[10]*

Adda Grimberg, MD

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Genomic Alterations in Human Embryonic Stem Cells

The potential use of human embryonic stem cells (hESC) is an exciting but controversial area in medicine today. In concept, hESC cells might be able to repair and/or regenerate damaged tissues and replace injured cells. It is often assumed that after harvesting, these cells are genetically stable, even though they must be expanded substantially through repeated cell division to generate enough cells for current experiments and for possible future therapeutic uses. However, like all dividing cells, it is probable that cultured hESC undergo a low level of spontaneous mutation, which in some cases could adversely affect their therapeutic potential. Maitra et al examined this issue by comparing several parameters of genomic stability in 9 hESC lines that were available as both early and late passage cells, ie, early and late passage paired cell lines. Cells normally stop dividing when they reach high density in culture, but they will start dividing again if diluted. Passage refers to this dilution process; it is a crude measure of the number of times cells have divided, ie, late passage cells have divided many more times than early passage cells.

The authors used 3 assays to search for alterations of cellular DNA: nuclear DNA copy number, mitochondrial DNA sequence, and gene promoter methylation. In the first case, initial Affymetrix high-density array analysis of approximately 115 000 single nucleotide polymorphisms

(SNPs) distributed across the genome showed no significant differences between the early and late passage hESC. However, further analysis revealed copy number alterations in late but not early passage cells from 4 of the 9 paired cell lines. The alterations ranged from large genomic regions of amplification or deletions, such as amplification of the entire chromosome 17q arm, to discrete changes such as a 2-Mb amplification that included the MYC oncogene. These changes were verified by *in situ* hybridization (FISH) or quantitative genomic PCR.

Next, they screened the mitochondrial genome, which is often mutated in cancer, again using array technology. Sequence alterations were detected in 2 of the late passage hESC cell lines that were not observed in early passage cells from the same cell lines.

Promoter methylation, an epigenetic phenomenon observed in almost all cancers, was assessed in a panel of 14 genes known to be differentially methylated in cancer cells. Differential methylation of 3 genes was detected in late passage cells. For one gene, RASSF1 – a putative tumor suppressor gene, increased methylation was found in late but not early passage cells from 7 of the 9 paired hESC lines.

In conclusion, the authors suggest that most but not all hESC lines maintained in cell culture acquire clonal DNA alterations over time. Many of these alterations are similar to what has been observed in cancer, such as loss of tumor suppressor genes or amplification of oncogenes. These alterations may provide a growth advantage that allows the cells that harbor them to dominate late passages. The authors acknowledge that much more work is needed to better define the nature of these alterations and their functional consequences. However, they argue that their findings underscore the need to periodically monitor hESC lines before they are used in *in vivo* applications and that some late-passage hESC may be unusable for therapeutic purposes due to genomic alterations over time.

Maitra A, Arking DE, Shivapurkar N, et al. Genomic alterations in cultured human embryonic stem cells. Nat Genet. 37:1099–1103.

Editor's Comment: *It is clear that hESC have great potential in regenerative medicine. However, this paper illustrates that the field is still relatively young with many troublesome issues, such as long-term genomic fidelity, must be resolved before it can be applied clinically.*

William A. Horton, MD

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In This Issue

GROWTH HORMONE AND MORTALITY IN PRADER-WILLI SYNDROME

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INTRODUCTION

Prader-Willi syndrome (PWS) is a unique condition associated with lack of normal expression of paternal alleles in a highly imprinted region of chromosome 15q11-13. Involvement of regions encoding small nucleolar RNA (snoRNA) clusters HBII52 and, perhaps more crucially, HBII85, have been identified as particularly associated with phenotype expression.[1,2] Minimum birth incidence has been recently estimated at ~1 in 20,000 to 30,000 and population prevalence at ~1 in 50,000 to 80,000.[3-5] Fewer than 1% of cases are due to inherited mutations.

Affected individuals suffer from excessive body fat independent of weight, marked deficits in muscle mass and function, growth failure with adult short stature, osteoporosis, scoliosis, hypogonadism, acromicria, neurodevelopmental delay, hyperphagia, and cognitive defects.[6] A variable deficiency of induced growth hormone (GH) secretion and more consistently-observed low insulin-like growth factor (IGF)-I levels are characteristic and may play a role in pathophysiology.[7]

In 2000, after nearly 15 years of favorable clinical experience, recombinant human (rh)GH became the first and, to-date, only pharmaceutical agent specifically approved for treatment of PWS. The FDA labeling states: "...for long-term treatment of pediatric patients who have growth failure due to PWS" and European

From The Editor's Desk

Yes, *GGH* has a new look to acknowledge a new era with our new sponsor, INSMED. Also, with this change we welcome 2 new distinguished colleagues to the editorial board, Dr. Roberto Lanes from Caracas and Dr. Martin Savage from London. Biographical sketches highlighting their wonderful credentials are available on the journal's website. The above mentioned changes have brought about a renewal of the journal's scope and mission that we hope will be appreciated and relished by the readers.

In this issue the lead article deals with an important current dilemma—the treatment of individuals with PWS with rhGH. Dr. Phil Lee was invited to review the mortality risks to these patients and his paper clearly presents the current status and issues. Although the data are not sufficient to fully determine patients' risks, with or without this therapy, the review was necessary. The lead article facilitates an understanding of the facts as they now stand and therefore it aids in formulating the clinical choices that need to be made when treating PWS. There clearly is a need to gather additional scientific information for a precise risk analysis in PWS that will lead to well substantiated recommendations.

The abstracts and editorial comments are also very timely, all dealing with a variety of subjects that affect patients and help elucidate important pathophysiological mechanisms ie, growth in Noonan syndrome and the role of Obestatin, a new hormone that opposes Ghrelin, among other important contributions. Altogether this issue constitutes another very successful issue of *GGH*; enjoy it and thank our new sponsor for their commitment to continuous medical education.

Sincerely,
Fima Lifshitz, MD
Editor-in-Chief

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labeling states "for improvement of growth and body composition" (Genotropin®/Genotonorm®, Pfizer, New York, NY); similar approvals have been obtained worldwide for other rhGH manufacturers. Numerous beneficial effects of rhGH, including improvements in linear growth, physical appearance, functional muscle mass, and infant neurodevelopment have been observed in children with PWS.[7-10] Treatment of adult PWS patients with rhGH is under investigation.[11]

In the nearly 20 years since the first reported use of rhGH in PWS, remarkably few adverse effects have been reported.[8,9] One case of intracranial hypertension[12] and a few cases of asymptomatic fluid retention[10] have been reported. Exacerbation of hyperglycemia and type 2 diabetes mellitus has been reported, usually with preceding risk factors.[8] Malignancy has not been reported with rhGH, although an increased risk for malignancy has been suggested for PWS without rhGH based on individual reports of various types of cancer[6] Neuromuscular scoliosis, a common progressive condition in PWS, is not worsened by rhGH.[6,10]

Two cases of death were reported in 2002 in children with PWS receiving rhGH.[13,14] On January 23, 2003, the Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society issued a statement (revised May 20, 2003) including 5 additional cases (www.lwpes.org). On April 30, 2003, Pharmacia (now, Pfizer) applied a warning label to its rhGH, Genotropin, followed by a letter to health care professionals dated May 30, 2003 (approved by the FDA on October 31, 2003) stating that "Growth hormone is contraindicated in patients with Prader-Willi syndrome who are severely obese or have severe respiratory impairment." In 2004, other manufacturers were required to add this warning to their rhGH products.

While the application of this warning was prudent in many respects, it has also led to considerable concern and confusion regarding the safety of rhGH in PWS. As a member of the Prader-Willi Syndrome Association USA (PWSA) Scientific Advisory Board, this author has been made aware of several cases in which rhGH has been denied or withdrawn by the treating physician because of this warning. Since denial of rhGH may be detrimental for children with PWS, it seems equally prudent to review the evidence for and against an association of rhGH with mortality in PWS.

MORTALITY DURING rhGH THERAPY

Following the initial reports of death during rhGH, intensive investigations for additional cases were conducted by rhGH manufacturers. As of February 2003, a total of 7 cases of death during rhGH treatment had been identified; 3 of these cases were previously registered in the Kabi International Growth Study (KIGS). At that time, a total of 675 rhGH-treated PWS cases were registered in KIGS (personal communication, Pfizer, October 3, 2003), giving an overall mortality ratio of 0.4%. Of the remaining cases in KIGS, 16% had only one recorded clinic visit while 84% (n=565) had received rhGH for a mean period of 2.4 years.

As of May 01, 2006, a total of 18 pediatric and 2 adult deaths have been identified in individuals with PWS treated with rhGH[13-19] (additional information from Dr. M. Wajnrajch, Pfizer and Dr. B. Lippe, Genentech). Considering the degree of attention given to this issue, it may be assumed that these cases represent a fairly comprehensive survey of deaths within the rhGH-treated PWS population throughout most of the industrialized world. (A database project conducted by PWSA may contain a few additional cases, as discussed in the next section.)

The 2 adult cases included a 33-year-old male who had been off rhGH for 6 weeks prior to his demise and a 48-year-old male who was known to be noncompliant with rhGH therapy. One pediatric case was a victim of bathtub drowning and another had been off rhGH for 11 months prior to death. These cases do not appear to be relevant to the current concerns. One case occurred in a 3-year-old who was known to be noncompliant with rhGH therapy; this case is included in the following analyses since therapy is not confirmed to have been discontinued for a significant period prior to death (case #8 in the Table).

As detailed in the Table, the 17 remaining cases were all pediatric, 0.7 to 15.8 years of age (7.0 ± 4.3 yr) (mean ± SD), including 13 males. Duration of rhGH ranged from 2 weeks to 2.5 years (0.57 ± 0.66 yr). Eight of 11 cases were known to be significantly overweight. The cases include 5 previously registered in postmarketing surveillance databases (KIGS-3, Genentech National Collaborative Growth Study [NCGS]-2), 1 case reported to Genentech (GEN), 4 investigated via a regulatory process known as Pharmacovigilance (PV), and 7 from the published literature and other sources. Therefore, approximately 60% of cases were detected via postmarketing surveillance databases or other reports to rhGH manufacturers, while 40% were not.

For the 16 cases for which data are available, the rhGH dose ranged from 0.10 to 0.33 mg/kg/wk (0.18 ± 0.06 mg/kg/week, mean ±SD). Twelve of 16 cases were receiving less than the labeled dose (0.24 mg/kg/week), 2 were at this level and 2 were above this level. The Figure depicts the doses for these cases and published doses from treatment series.

For the 9 cases in which a possible contributory factor is listed, respiratory illness was listed in all cases. Eight of 17 cases were characterized by "sudden" death. Six of these had respiratory impairment preceding rhGH therapy, one

Table. Deaths During GH Treatment

Case	Year Reference*	Age(yr) Sex	Country	Duration rhGH (yr)	Dose rhGH (mg/kg/wk)	Weight***	Cause of Death****	Comments
1	1996 (KIGS)	15.8 M	Japan	0.58	0.10	BMI=46.6	acute pneumonia, respiratory failure; no autopsy	
2	1999 (NCGS)	6.8 M	USA	0.50	0.23	BMI=23.7	died in hospital no autopsy	cardiomegaly, on carbamazepine and O_2
3	2001 (PV)	3 M	USA	0.25	0.5 mg qd** (~0.15)	>200% IBW	found dead in bed; autopsy: ? pneumonitis	asthma-on albuterol;
4	2001 (KIGS)	8-9 M	Spain	0.04 (2 wks)	0.15	BMI=38.5	acute bronchitis, respiratory failure	history of OSA, nocturnal hypoventilation
5	2001 [14]	0.7 M	Switzerland	0.20	0.18	WT 0.63SD	aspiration pneumonia; autopsy: bronchopneumonia	died 2 days after aspirating milk
6	2001 [13]	6.5 M	Switzerland	0.50	0.26	145% IBW	found dead in bed	history of snoring, OSA, large tonsils
7	2002 (KIGS)	4.7 M	USA	0.25	0.24	BMI =31.3	aspiration pneumonia, sleep apnea; no autopsy	history of OSA
8	2003 (PV)	3 F	USA	2.50	0.5 mg qd** ~0.18 noncompliant	BMI= 17.6 at 2 yr (~75th percentile)	pneumonia	history of aspiration pneumonia
9	2003 (PV)	13 M	UK	0.42	no data	overweight	sudden death, unexplained	
10	2003 (PV)	14.6 M	UK	1.50	0.11	BMI=42.0	viral respiratory infection, respiratory and right heart failure	
11	2003 [19] (NCGS)	4.5 M	Canada	0.17	0.17	259% IBW	died during sleep; autopsy: pneumonia, left ventricular hypertrophy, subdural hematoma	history of progressive snoring, headaches
12	2003 (GEN)	10 M	USA	~0.13	0.15	BMI= 51.6	abrupt deterioration	history of albuterol use
13	2005 [16]	4.7 F	Austria	~0.13	0.24	BMI=19.5	abrupt deterioration, ? cardiorespiratory arrest at home	previous adenoidectomy; no apnea on PS; nocturnal NCPAP
14	2005 [16]	9.3	Austria	1.0, stopped 1.3, restart 0.5; total treatment= 1.5 yr	0.28, restart 0.14	BMI 30.2, 27.3 after 1st rhGH, 38.5 at restart	minor respiratory infection, sudden death at home	Progressive deterioration after stopping first course of rhGH; PS-hypoventilation & apnea, noncompliant with CPAP
15	2005 [17]	3.9 F	Italy	0.30	0.33	130% IBW	sudden death, morning	adenoid hypertrophy, snoring, apnea preceding therapy
16	2005 [17]	6.3 M	Italy	0.20	0.20	144% IBW	sudden death, morning apnea	TA hypertrophy, respiratory impairment preceding rhGH, worsened during treatment
17	2005 [18]	3.9	Greece	0.58	~0.10	Severe obesity	sudden death	
Mean ± SD		7.0 ± 4.3		0.57 ± 0.66	0.18 ± 0.06			

Notes: OSA=obstructive sleep apnea, PS=polysomnography, TA=tonsillo-adenoidectomy, NCPAP=nasal CPAP
*Year of death, if known, or publication. Source: Kabi International Growth Study (KIGS), National Cooperative Growth Study (NCGS), Pharmacovigilance (PV, Pfizer), 1 case reported to Genentech (GEN)
**Weight at time of death or rhGH dose per kg were not available for these 2 cases. For the purposes of analysis, the weights in both cases were assumed to be 20 kg, giving approximate rhGH doses of 0.18 mg/kg/wk in each case.
***%IBW= percent ideal body weight. All BMI calculations are >97th percentile for age and sex, except as indicated.
****Causes of death as reported to database are usually based on clinical reports. Autopsy findings are indicated if available.
For case 14, total treatment duration of 1.5 yr and the dose at time of death (0.14) were used for the analyses in the text.

case was reported to have worsened while on rhGH (case 15), while most of the others had no known change during therapy. Inadequate details were available for several cases, and one individual was said to have been improving on rhGH without known respiratory problems (case 17). Twelve cases were known to be morbidly overweight (>200% IBW or BMI >95th percentile for age and sex), while 5 cases (5, 6, 8, 15, and 16) were apparently within normal weight guidelines for height. Autopsy data was notably lacking for most cases, and the available data do not reveal unexpected findings. Large tonsils were noted for case 6, but this was not thought by the authors to be contributory to death.[13] Adrenal abnormalities have not been noted in the autopsied cases.

MORTALITY WITHOUT rhGH THERAPY

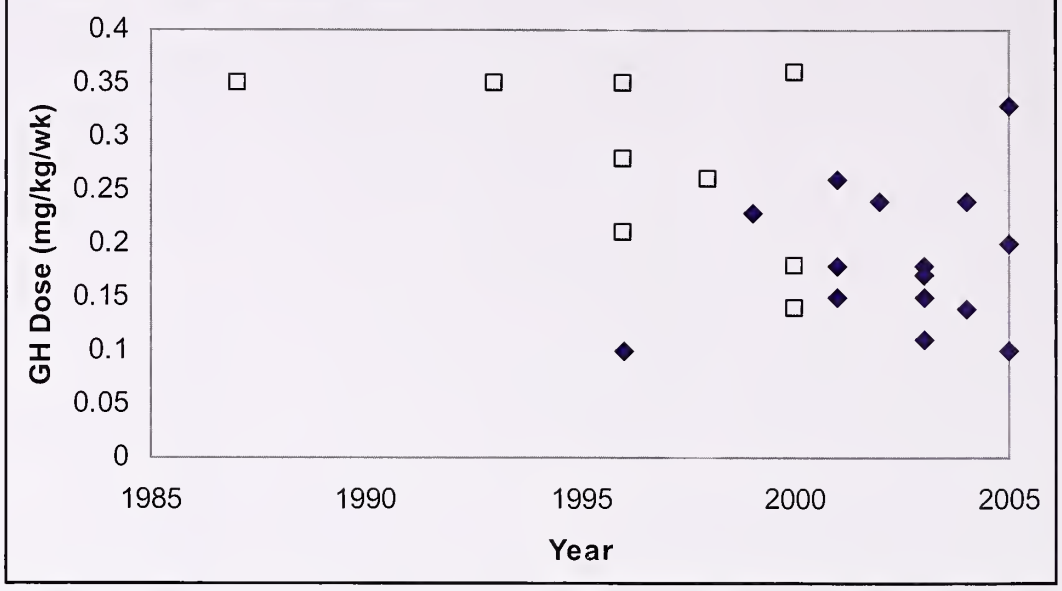
Premature mortality and sudden death in PWS are not new concerns; these predate rhGH treatment of PWS by many years.[20] In 1981, Laurance[21] reported a series of 33 patients, of whom 24 were alive at 15 to 41 years of age, and 9 of whom had died before age 23 years. The deaths were attributed to cardiorespiratory failure.

A retrospective clinical review of 36 individuals with PWS found 10 deaths (20-49 years old) over a 10-year period.[22] Respiratory or cardiorespiratory illness was identified as causative in 40% of cases. In 2003, 6 deaths (20-43 years old) were tabulated in a follow-up study of 37 non-rhGH treated individuals with PWS who had been entered into

the Australian Child Development Study in 1989.[23] The calculated death rate was >4-fold higher than in a control group of 547 individuals with intellectual disability drawn from the same prospective study. A respiratory component was noted for 3 of the 4 cases for which cause-of-death was identified.

In an international summary of 27 deaths in PWS,[24] approximately half were related to respiratory or cardiorespiratory disease, including 9 of the 13 cases in those less than 5 years of age. Small adrenals were not observed in autopsied cases, but were noted in 3 of 4 autopsied cases in a separate review of 10 cases.[25] It should be noted that no functional adrenal abnormalities have been identified in PWS patients on standard biochemical testing.[6]

Figure. GH Dose versus Year



Empty squares: rhGH doses reported in published series demonstrating rhGH efficacy.[7,9] **Closed diamonds:** Individual cases of death while on rhGH, plotted by dose at time of death and year reported (see Table).

Support-group survey studies are limited by substantial bias, including survival and younger age. However, by virtue of their size and geographic representation, these efforts may provide valuable information regarding the characteristics of the PWS population. Nagai et al[26] examined the records of 494 individuals with PWS registered in 2 regional PWS support groups (2 months to 48 years of age, 46% females, 54% male). Thirteen deaths were identified (2.6% of the group, 6 female, 7 male), none had received rhGH; 7 (58%) were under 2 years of age, with deaths attributed to possible aspiration or SIDS (n=2), upper airway infection with diarrhea (n=1), cardiomyopathy with known respiratory disorder (n=1), and diarrhea (n=3). None of the infant deaths were associated with obesity. Other deaths included a 14-year-old and 20-year-old with bathtub drownings. The 4 adult deaths (23, 26, 28 and 34 years old) were attributed to cellulitis, pulmonary embolism, renal, and heart failures, respectively.

An ongoing survey of the PWSA membership (approximately 3000) has thus far revealed 190 deaths since 1977 (74% dated since 2000 [courtesy of J Heinemann, PWSA]). Mean age at death was 28 years (2 months to 63 years, 33% <21 years, 12.5% <5 years). The 14 cases reported to have received rhGH included 4 who were off therapy for several months or years, 3 with gastric perforation–a known cause of mortality in PWS,[27] (3 without information, and 1 each: motor vehicle accident, sepsis, severe asthma attack, and possible poor intubation (pre-existing respiratory problems). None of the cases appear to be related to rhGH, although complete analyses for some cases are pending.

Population-based morbidity and mortality data for PWS are not available except from regional cross-sectional surveys. Recent regional surveys in England[5] and Belgium[4] indicate high morbidity and mortality rates; survival past the 6th decade of life has been rarely documented. In the English study,[5] 50% of individuals with PWS reported recurrent respiratory disease, and lifetime mortality rate was roughly estimated at 3%/yr (approximately 3 times higher than the general population). Except for these 2 population-based studies, no conclusions can be reached regarding mortality rates, and within these studies the data are insufficient to construct survival curves.

IS MORTALITY INCREASED WITH rhGH THERAPY?

Given the similarity in causes of death between the rhGH-treated and untreated cases and the apparently high underlying mortality rate at all ages in the untreated population, a logical question is whether rhGH is having a positive, negative, or neutral effect on mortality risk in PWS. The answer to this question is complicated by the lack of sufficient population-based data to construct survival curves or risk ratios. In addition, little is known about the effects of age, sex, and accompanying morbidities on mortality; information that would be crucial for estimating the additional effect of rhGH treatment.

Moreover, most clinicians are not personally familiar with the natural history of PWS, have not cared for PWS patients as a series, and are unlikely to systematically follow patients who are not rhGH-treated. There are few centralized PWS care facilities from which experiential information can be collected. Since deaths without rhGH do not engender the same level of interest as those occurring during therapy and clinical experience is limited, the casual reader or incidental PWS practitioner may have the impression that the latter represent a new and unusual series of events.

Given the lack of rigorous statistical data for epidemiologic analyses, logical models of disease causation can provide an alternate framework for consideration. For instance, at least 3 of the Evans criteria[28] (paraphrased for the current discussion), originally formulated for infectious diseases but often applied to other cause-and-effect associations, appear to be in doubt:

1. The prevalence of death should be significantly higher in those treated with rhGH than in those not treated:

   As noted above, as of February 2003, mortality occurred in 0.4% of 675 rhGH-treated PWS cases registered in KIGS. Although this information has not been recently updated, using a conservative estimate that 1000 PWS patients have received rhGH for more than 1 year over the past 15 years and 20 deaths occurred during therapy, the death rate would be <0.2%/year. This compares to the 2.6% mortality ratio,[26] and 2.8% and 3%/year mortality rates[5,22] estimated for untreated PWS individuals. Therefore, although the available data are not perfect, these suggest that mortality may not be higher in those treated with rhGH.

2. There must be a certain strength of association, eg, duration, dose-response relationship:

   Higher doses do not result in increased mortality; 70% of cases were receiving rhGH doses below the labeled recommendation of 0.24 mg/kg/wk, while the published literature indicates that major PWS treatment centers are using the labeled or higher doses (Figure). In addition, higher doses were not associated with shorter duration of therapy prior to death.

   Continued exposure to rhGH apparently does not continue or increase the risk of mortality. The 17 cases (Table) received rhGH for an average of 6 months (median 4 months, 2 weeks to 2.5 years). As of February 2003, the average treatment duration for 565 PWS patients in KIGS was 2.4 years, and we can assume that the numbers receiving therapy for extended periods has increased since then. However, there is no evidence for increasing numbers of deaths during longer-term therapy. In fact, the paucity of reported deaths after 1 year of rhGH provides suggestive evidence for rhGH-related reduction of the high underlying mortality rate in PWS.

   Arguments have been made that the apparent early clustering of deaths represents a time-limited risk of rhGH therapy. For instance, there could be a dual effect of higher mortality in the initial phase of rhGH treatment and lower mortality thereafter, although a mechanism for the initial-phase effect has not been elucidated.

   It is also possible that these 17 cases represent continuation of the natural history of the condition. At standard doses, positive effects of rhGH on respiratory parameters are particularly evident after 6 to 12 months of therapy; thereby providing a window during the early phase of treatment during which natural history may take precedence. The situation may be as suggested in the first report: "The boy reported here...thus died before the effects of rhGH could manifest themselves."[13]

3. A coherent association should exist between rhGH treatment and death; the cause-and-effect interpretation should not conflict with the known pathology of the disease:

   For the 17 cases (Table), the most commonly identified disease at time of death was respiratory failure, which is also the most commonly identified mortality association in the non-rhGH treated PWS population. There is no evidence that rhGH worsens risk for respiratory-related morbidity. In fact, rhGH has been shown to improve pulmonary function and respiratory control in PWS.[29-31] Since excess GH levels are associated with respiratory complications in acromegaly, it has been postulated that rhGH could cause similar problems in PWS. However, such complications in acromegaly are complex and thought to be due to a combination of soft-tissue and bone remodeling,[32] changes which have not been observed in rhGH-treated pediatric populations. In addition, one might expect acromegalic airway changes at higher rhGH doses and with longer duration of therapy.

POLYSOMNOGRAPHY AND rhGH THERAPY

The involvement of respiratory compromise in the initial 7 cases of death during rhGH therapy prompted the manufacturer in April 2003 to expand the warning label on Genotropin to include: (1) severe respiratory impairment as a contraindication to therapy, (2) worsening "upper airway obstruction," including snoring, as an indication for interruption of therapy, and (3) evaluation and monitoring for sleep apnea. No statistical data in support of this sternly-worded warning label and no specific methods for assessment were presented. The result was clinical practice and liability concerns amongst clinicians accompanied by alarm amongst parents of children with PWS, primarily concerned that an approved and beneficial treatment would be withheld from their child. Many clinicians interpreted this label to mean that all children with PWS should have polysomnography and that rhGH should be withheld upon receipt of abnormal results. This was despite the fact that no relationships between polysomnographic results and morbidity or mortality in children with PWS had been identified, a population in which 0% to 100% occurrence of obstructive sleep apnea had been reported in various series.[33]

After careful consideration of all available data and viewpoints, the Clinical Advisory Board of PWSA issued reasoned recommendations for sleep studies and other testing in 2003 (www.pwsausa.org).[6] As stated: "At this time

there is no evidence of a causative link between growth hormone and the respiratory problems seen in PWS."
Several studies have shown improvements in breathing and pulmonary function in children with PWS after 6 to 12 months of rhGH.[6,8,9,29-31] Over a much shorter rhGH treatment period of 6 weeks in a mixed group of children and adults with PWS, 19 of 25 (76%) had improved polysomnographic parameters.[34] A non-treatment control group was not studied and test/re-test reproducibility was not reported. Nonetheless, this latter study indicates that rhGH efficacy might be observed even over a short term. Also, in this latter study, IGF-I levels were noted to be high in 2 subjects with worsened parameters, leading the authors to postulate a role for rhGH/IGF. However, the other 4 subjects with deteriorating measures had normal IGF-I levels, and 2 subjects in the improved group had high IGF-I levels.

As of this writing, there is no evidence linking results of polysomnography with morbidity or mortality in PWS, regardless of rhGH therapy. Whether an abnormal polysomnogram itself defines morbidity is a matter for debate that is beyond the limits of this manuscript. This author concurs with recommendations that polysomnography be reserved for individuals with clinical evidence of sleep-disordered breathing or excessive daytime sleepiness, and should be preferably performed as part of a clinical research program in other cases.[6,33] Similar guidelines may be logically applied to pulmonary function testing.

CONCLUDING PERSPECTIVES

If left untreated, PWS can be a devastating condition, with affected individuals suffering considerable physical handicap, largely related to severe lifelong hypotonia. The efficacy of rhGH, particularly in children with PWS, has provided a new outlook on life that goes beyond obvious improvements in height and somatic appearance. Against these recognized benefits are concerns that rhGH may increase mortality in the initial phase of therapy. Although conclusive data supporting or refuting this concern may or may not be available in the near future, the bulk of information reviewed above may serve as an argument against the validity of this concern.

Based on the available information, rhGH may be considered in children with PWS with prudent consideration of the following points:

1. Many deaths in infants with PWS, regardless of rhGH therapy, have been related to possible aspiration of feedings. Therefore, reflux precautions should be stringently followed until the child is ambulatory.
2. Many deaths in older children and adults with PWS, regardless of rhGH therapy, have been associated with obesity; albeit without direct demonstration of cause/effect in most cases. In addition, rhGH can exacerbate the insulin resistance associated with being overweight. Therefore, proper attention should be given to weight control.
3. Several tub-drowning deaths have occurred in individuals with PWS, regardless of rhGH treatment. Caretakers should be warned not to leave individuals with PWS unattended in a bathtub or pool.
4. Most of the reported deaths during rhGH treatment occurred with doses at or below the labeled recommendation of 0.24 mg/kg/week. Therefore, there is no apparent reason to limit the rhGH dose in relation to preventing morbidity or mortality.
5. All but one of the reported deaths during rhGH therapy occurred within the first 18 months of treatment; 82% within the first year. Therefore, clinical follow-up should be especially attentive during the first 12 to 18 months of rhGH. For patients who are not receiving rhGH, this heightened level of attention should be continual given the high inherent mortality rate.
6. There is currently no medical reason for rhGH to be conditional on the results of polysomnography or pulmonary function testing. Such testing should be considered only if clinically indicated and/or within the guidelines of a clinical research protocol.

Unfortunately, a current lack of population-based data regarding mortality and rhGH therapy in PWS prevents conclusive analyses, such as survival curves and hazard ratios, which are required to define therapeutic risk. However, assessment of current available information argues against a cause and effect relationship between rhGH treatment and mortality. Coordinated multicenter studies of treated and untreated populations are needed to bring closure to this issue. Meanwhile, in keeping with the principles of *primum non nocere* and the Doctrine of Double Effect,[34] each clinician involved with decisions regarding rhGH therapy of PWS should maintain an awareness of current knowledge regarding therapeutic efficacy, natural history and adverse effects to insure optimal care of individual patients.

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DISCUSSION OF UNLABELLED USES: none

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ABSTRACTS FROM THE LITERATURE

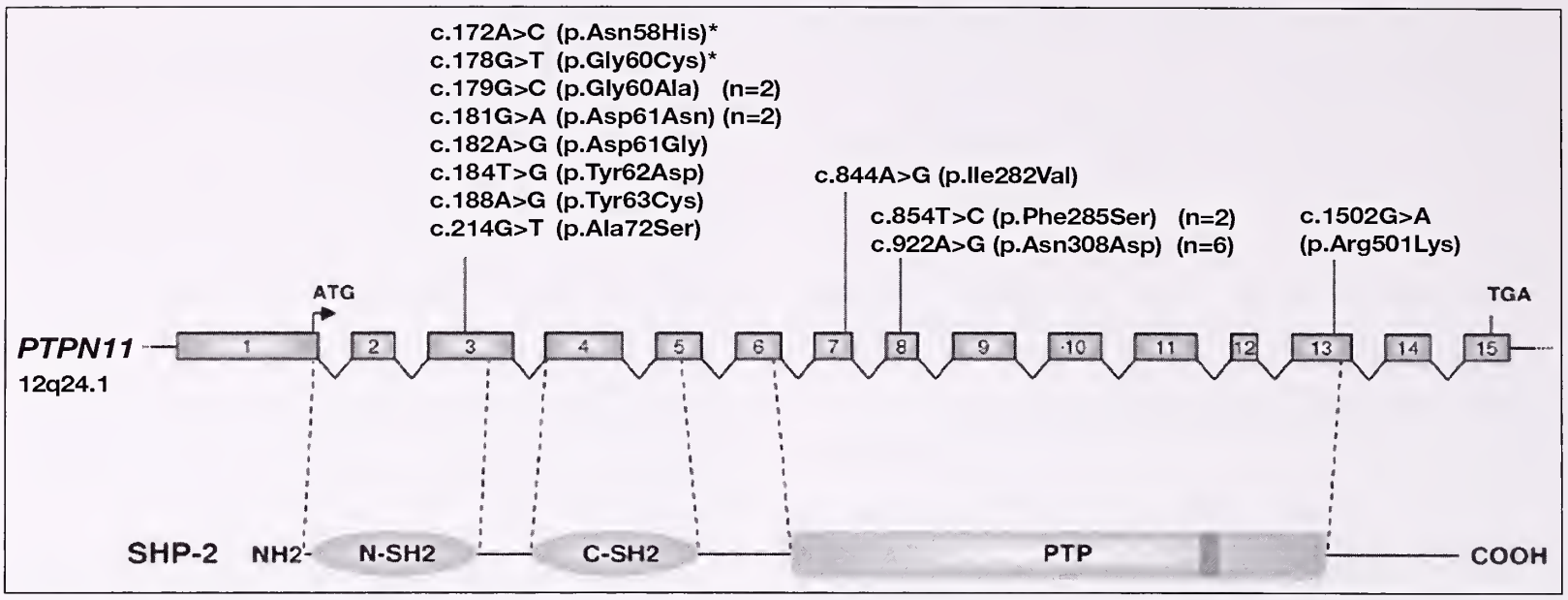
GH Resistance in Noonan Syndrome: From Cause to Clinical Outcome

Proportionate short stature (SS) occurs in more than 70% of individuals with Noonan syndrome (NS), an autosomal dominant disorder found in 1:1000 to 1:2500 live births. NS is also characterized by typical facial dysmorphisms and cardiac defects, especially pulmonic stenosis and hypertrophic cardiomyopathy. Although prior growth hormone (GH) studies in these patients have shown mixed results (some normal, some abnormal, some suggesting neurosecretory deficiency), in general classic GH deficiency is a rare finding.

A causative gene for NS was identified in 2001: *PTPN11 (protein tyrosine phosphatase, nonreceptor type 11)*, which encodes Src homology region 2-domain phosphatase-2 (SHP-2). About half of individuals with NS harbor heterozygous missense mutations of SHP-2, the majority of which involve the amino SH2 (N-SH2) or the protein tyrosine phosphatase (PTP) domains (exons 3,8, and 13). Both N-SH2 and PTP normally interact, keeping the ubiquitously expressed, cytosolic SHP-2 in a closed, inactive conformation. SHP-2 is activated upon binding of N-SH2 to a phosphotyrosine residue, such as those on activated receptors for GH, cytokines and other growth factors. By chronically stabilizing the SHP-2 open, and hence active, conformation, the missense mutations of NS would be expected to cause gain of function of this negative regulator of receptor signaling. SHP-2 can not only dampen signaling through dephosphorylation of the receptor itself, it can also dampen downstream signals like dephosphorylating STAT5. Thus, SHP-2 mutations would be expected to cause GH resistance in patients with NS. Three recent papers studied this proposed hypothesis.

Mild GH Resistance

Binder and colleagues recruited all 29 children who presented to their center during the past 5 years with SS and at least 3 typical anomalies of NS or pulmonic stenosis. Blood lymphocyte DNA was extracted for PCR amplification and sequencing; 11 different missense mutations of *PTPN11* were found in 16 children from 14 unrelated families (55% of patients). Of these 11 mutations, 8 occurred in exons 3, 8 or 13. Comparing the mutation-positive (mut^+) vs mutation-negative (mut^-) subgroups, the former were found to have a higher incidence of pulmonic stenosis (81% vs 15%) and septal defects (63% vs 15%), and younger mean age at presentation (5.1 ± 2.7 vs 10.3 ± 5.2 years). Minor anomalies and height (–3.15 ± 0.92 vs –3.01 ± 1.35 SD) did not differ significantly, and all children were approximately 1 SD shorter in height than the mean for NS. While the higher spontaneous overnight and arginine-stimulated GH levels did not reach statistical significance, insulin-like growth factor (IGF)-I (–2.03 ± 0.69 vs –1.13 ± 0.89 SD) and IGF binding protein (BP)-3



Distributioin of *PTPN11* missense mutations identified in 20 of the 35 NS patients. Mutations that have never been described are marked by an asterisk. The number of patients carrying the same mutation is indicated in *parentheses*.

(–0.92 ± 1.26 vs 0.40 ± 1.08 SD) were significantly lower in the mut$^+$ group.

A subgroup of 11 prepubertal children received recombinant human (rh) GH for one year. Mean change in height SDS in the 8 mut$^+$ children (+0.66 SD) was significantly lower than that in the 3 mut$^-$ children (+1.26 SD). However, the mut$^+$ children received a lower mean rhGH dose (0.042 mg/kg/d vs 0.05 mg/kg/d).

Binder G, Neuer K, Ranke MB, Wittekindt NE. PTPN11 mutations are associated with mild growth hormone resistance in individuals with NS. J Clin Endocrinol Metab. 2005;90:5377–5381.

Response to 3 Years of rhGH Treatment

Ferreira and colleagues retrospectively analyzed the 14 children (10 male) followed at their Endocrinology Unit for NS; all had presented with SS (mean height –3.5 ± 0.9 SD) and were treated with (0.033–0.05 mg/kg/d) after a 6-month observation of baseline growth velocity. Eight of the children had been treated for 3 years, 4 for 2 years, and 2 for at least 1 year at the time of analysis. At the start of treatment, mean age was 12.3 years, bone age 9.8 ± 2.7 years, and 10 were prepubertal. Seven children initiated puberty during treatment, and one received concomitant gonadotropin releasing hormone (GnRH) analog therapy. Treatment with rhGH was discontinued during the second year in one patient for increasing ventricular wall thickness; this patient had mild left ventricular hypertrophy before starting rhGH, and cardiac function continued to worsen afterwards despite cessation of rhGH.

Gene sequencing revealed 5 different, *de novo* heterozygous *PTPN11* missense mutations in 7 (50%) patients, 3 of whom were also seen among the children in the above Binder paper. At the start of treatment, the 7 mut$^+$ and 7 mut$^-$ patients did not differ in their GH secretory capacity (all had normal peak responses to clonidine stimulation; mean 13.1 ± 7.1 ng/mL), nor in their low IGF-I levels (–2.0 ± 1.4 SD). However, the rhGH-stimulated increment in IGF-I was significantly smaller in the mut$^+$ patients, as was the improvement in growth velocity, such that by the end of the third year of treatment, the mut$^+$ group had a significantly smaller gain in height SDS (+0.8 ± 0.4 vs +1.7 ± 0.1 SD; $p<0.01$). Bone age advancement did not differ between the 2 groups.

Ferreira LV, Souza SA, Arnhold IJ, Mendonca BB, Jorge AA. *PTPN11* (protein tyrosine phosphatase, nonreceptor type 11) mutations and response to growth hormone therapy in children with NS. J Clin Endocrinol Metab. 2005;90:5156–5160.

Prospective Study of 2 years of rhGH Treatment

Limal and colleagues prospectively recruited 35 patients (19 boys) with NS and growth retardation (height <–2 SD), excluding those with severe congenital heart malformations and/or hypertrophic cardiomyopathy. The 25 prepubertal children at study start (mean age 10.4 ± 3.1 yr) were given rhGH 0.30 mg/kg/wk while the 10 pubertal children (mean age 14.7 ± 1.7 yr) were given rhGH 0.46 mg/kg/wk to compensate for their late treatment start.

Sequence analysis revealed 12 different heterozygous *PTPN11* missense mutations in 20 children (57%) (Figure), 10 of which were previously reported; all but one occurred in exons 3, 8 or 13. The mut$^+$ subgroup had a higher frequency of small-for-gestational age (SGA [32%]) than the mut$^-$ (13%), though birth weight and head circumference were normal in all. At age 6 years, the mut$^+$ group was significantly shorter, as was their mean target height. Starting at age 10.4 ± 3.1 years, 2 years of rhGH resulted in less catch-up growth among the prepubertal mut$^+$ children than the prepubertal mut$^-$ children; their end heights were –3.1 ± 1.4 SD (vs –2.0 ± 0.9 SD; $p<0.05$) and deficit from target heights were –2.5 ± 0.9 SD (vs –1.1 ± 0.7 SD; $p<0.01$).

At initiation, peak GH level following pharmacologic stimulation was 15.4 ± 6.5 ng/mL (5 –34.3) in all 35 children, though 5 of the mut⁻ had peaks of 5 ng/mL to 10 ng/mL. Of the 19 patients studied (11 mut⁺ and 8 mut⁻), all had normal IGFBP-3, but they had IGF-I at or below the lower limit of normal, and acid-labile subunit (ALS) levels were extremely low in all 10 patients (5 mut⁺) tested at rhGH initiation. There was no difference between the 2 genetic subgroups in rhGH-stimulated increases in IGFBP-3 and IGF-I.

Limal JM, Parfait B, Cabrol S, et al. NS: Relationship between genotype, growth and growth factors. J Clin Endocrinol Metab. 2006;91:300–306.

Editor's Comment: *These 3 related papers offer intriguing glimpses into a possible mechanism of growth failure in NS. There are clearly additional mechanisms involved, since mut⁻ patients frequently also have SS. Nonetheless, as a group, these papers suggest new directions.*

Mechanism

In the idiopathic SS age of non-GH deficient growth failure, the quest has been on for molecular causes of post-receptor GH resistance. The search for individuals who harbor mutations in the signaling cascade directly downstream of the GH receptor has yielded fruitful results: novel GH receptor mutation that impairs GH receptor/STAT5 signaling but maintains normal STAT3 signaling,[1] mutations of STAT5b itself,[2] IGF-I gene partial deletion,[3] singe copy number of the IGF-I gene,[4] and IGF-I receptor mutation.[5]

Yet these papers on NS serve as a reminder that a signaling cascade can be turned off (or down) not just by mutations from within, but also by mutations affecting molecules from without; gain of function mutations of negative regulators of a cascade, such as SHP-2, can serve to augment the normal checks and balances and overly suppress the signaling cascade. This is not the first time that such possibility was shown. In 2001, 8 years after the FDA approved rhGH treatment for SS associated with chronic renal insufficiency, the molecular mechanism underlying the GH resistance was discovered. Comparing rats status-post partial renal ablation (chronic renal failure) and sham-operated, pair-fed rats (controls), Schaefer and colleagues found the former to have blunted hepatic induction of IGF-I expression by GH treatment despite unchanged GH receptor protein levels and GH binding to microsomal and plasma membranes.[6] Normal protein levels of JAK2, STAT5, STAT3, and STAT1 completed the cascade. Instead, these authors[6] found a 75% reduction in GH-induced tyrosine phosphorylation of JAK2, STAT5, and STAT3, due to over-expression of SOCS (suppressor of cytokine signaling)-2 and -3. The SOCS proteins normally function as a cellular internal feedback loop; they are induced by GH and in turn, inhibit GH-stimulated GH receptor/JAK2 complex activation to turn down the GH sensitivity of the cell.

The over-expression of SOCS in chronic uremia and the gain of function mutations of SHP-2 in NS may be just the beginning. Further search may reveal additional conditions involving augmented negative regulators, as well as loss of positive stimulators and enhancers, of the GH receptor/JAK/STAT signaling cascade. Thus, the quest for non-GH deficient causes of growth failure just got a whole lot broader.

Clinical Implications

The increased GH resistance of PTPN11 mut⁺ vs mut⁻ patients reported in these papers suggests that a genotype-driven approach may be more effective for ameliorating the SS associated with NS. Two treatment strategies may be plausible, and additional studies designed to test these approaches will be needed to determine their relative efficacies and safety. First, to overcome the increased GH resistance, rhGH therapy may require higher doses, and an approach titrating rhGH dose to achieve desired IGF-I levels rather than a standard weight-based dosing scheme, may be the best way to gauge clinical requirements of mut⁺ vs mut⁻ individuals. Thus, we may discover 2 different

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optimal dosing levels based on genetic subtype. On the other hand, we may discover that the degree of GH resistance in the mut⁺ individuals is so great that cranking up the rhGH dose really cannot compensate effectively or may be associated with undesirable side effects. In this scenario (the second treatment strategy), treating with recombinant IGF-I and/or IGF-I/IGFBP-3 rather than rhGH, may be more appealing. These therapies have now become available and were recently approved by the FDA.

Adda Grimberg, MD

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Signal Transduction and Cardio-Facial Syndromes

The cardio-facio-cutaneous (CFC) syndrome (OMIM 115150) presents with heart malformations, skin defects, and characteristic facies. It overlaps phenotypically with Noonan syndrome (NS) and Costello syndrome (CS). Gain-of-function mutations have been identified in the protein tyrosine phosphatase SHP-2 (PTPN11) in about half of patients with NS. Recently, mutations of one of the RAS proteins known as HRAS were identified in several patients with CS. Interestingly, several CS mutations had been previously identified as somatic oncogenic mutations in tumors. SHP-2 and HRAS are components of a well-known signaling cascade through which many receptor tyrosine kinases transmit signals to the nucleus. Illustrated in the figure, this pathway, which is often referred to as the RAS-MAP kinase pathway, is often associated with proliferative and growth signals in developing tissues and in cancer.

Based on the suggestion that NS and CS might reflect activation of this pathway, a group headed by Aoki speculated that CFC syndrome might be due to mutations in genes encoding other proteins in this cascade. They first sequenced the entire coding regions of 3 *RAS* genes (*HRAS*, *KRAS*, and *NRAS*) in genomic DNA from 43 individuals with CFC syndrome. Two *de novo KRAS* mutations were detected.

Next, they screened for mutations in the 3 isoforms of RAF (CRAF, BRAF, and ARAF), which is immediately downstream of RAS in the signaling cascade. Eight *BRAF* mutations were identified in 16 patients, 6 of which mapped to the kinase domain, where mutations had previously been found in tumors.

The investigators proposed that the mutations they had identified enhance MAP kinase signal activity and tested this notion by expressing the mutant genes and their normal control counterparts in reporter cells that would allow downstream signal output to be measured. They observed a significant increase in signal output for 1 of the 2 *KRAS* mutations and in 4 of the 8 *BRAF* mutations, supporting their contention and the idea that increase MAP kinase signaling is common to all of the disorders in this group.

They reasoned further that if all of the disorders share a common increase in RAS-MAP kinase signaling activity, then there may be mutational overlap as well. Accordingly, they screened for *BRAF* and *KRAS* mutations in *PTPN11*-negative NS patients and for *PTPN11* mutations in CFC patients negative for mutations in *BRAF* or *KRAS*. No additional mutations were detected, suggesting that the 3 disorders are distinct entities.

In an accompanying editorial,[1] it is noted that a recent publication identified *BRAF* mutations in 18 of 23 individuals with CFC. This study also found mutations in MAP2K1 and MAP2K2, which are downstream effectors of BRAF in the RAS-MAP kinase signal pathway. The editorial also points out that molecules in which mutations have been found typically participate in other signaling pathways in addition to the primary linear RAS-MAP kinase pathway, which probably explains why each syndrome has unique features.

Niihori T, Aoki Y, Narumi Y, et al. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat Genet. 2006;38:294–296.

Editor's Comment: *MAP kinase signaling pathways are more complex than suggested in the figure, and there is extensive crosstalk between subpathways. Nevertheless, placing these syndromes into a group that results from enhanced RAS-MAP kinase signaling serves a useful purpose, especially as inhibitors of this pathway might potentially have therapeutic benefit for postnatal manifestations of these disorders, such as short stature.*

In contrast to most cell types in which RAS-MAP kinase signaling is associated with cell proliferation and growth, such signals in growth plate chondrocytes, where they are generated downstream of FGFR3, inhibit both cell proliferation and growth. Thus, it is conceivable that achondroplasia, which is due to activating mutations of FGFR3, NS, CS, and CFC syndromes share a common pathogenetic mechanism that involves excessive output of the RAS-MAP kinase signaling cascade in growing bone.

William A. Horton, MD

Reference

1. Editorial. Nat Genet. 2006;38:267.

Defective Enzyme Degradation in Johanson-Blizzard Syndrome

The Johanson-Blizzard syndrome (JBS [OMIM 243800]), names not unfamiliar to *GGH* readers, is an autosomal recessive disorder characterized by congenital exocrine pancreatic insufficiency, mental retardation, facial abnormalities, and various other malformations. As reported by Zenker et al, an international group has now identified the mutant gene. They started by undertaking a genome-wide linkage scan of 7 families with JBS. Analysis of the consanguineous families revealed a region of homozygosity of 7.5 cM on chromosome 15q14-21.1 containing no obvious candidate genes. By high-throughput DNA sequencing of genomic DNA from JBS patients, they eventually detected mutations in the gene *UBR1* in patients from 12 unrelated families. Most of the mutations produced premature translation stop codons and most likely loss-of-function for the gene product.

UBR1 encodes an E3 ubiquitin ligase, which transfers ubiquitin moeties to proteins destined for degradation by cytoplasmic proteosomes. Long chains of ubiquitin serve as molecular signals that direct targeted molecules for degradation. *UBR1* was one of the first of many E3 ubiquitin ligases to be identified; each possesses specificity regarding which molecules it ubiquitinates. Interestingly, several E3 ubiquitin ligases have been implicated in genetic disease, ie, UBE3A in Angelman syndrome, parkin in recessive juvenile parkinsonism, and VHL in von Hipple-Lindau disease.

To explore how loss of *UBR1*—which would be expected to lead to failure to ubiquitinate proteins normally ubiquitinated by *UBR1*—causes JBS, the investigators focused on the exocrine pancreas, since it is the most consistently affected organ system in JBS. Examination of pancreatic tissue from 2 fetuses and a newborn infant with JBS showed loss of acinar tissue with inflammation that worsened with gestation, suggesting a gradual destruction resembling pancreatitis as the fetus approaches term.

They next turned to *UBR1* null mouse model. These mice were viable and fertile, but display reduced weight with a proportionate decrease in both muscle and adipose tissue. Their feces contained reduced amounts of chymotrypsin and elastase, indicating pancreatic exocrine insufficiency. They next documented that compared to controls, exocrine cells cultured as acini-like structures exhibited a marked reduced response to treatment with cholecystokinin, the physiologic secretogogue of the exocrine pancreas. The investigators speculated that levels of pancreatic exocrine proenzymes and/or their derivatives may normally need to be kept in check by proteolytic degradation in proteosomes, and that this fails to occur in JBS. Similarly, they suggested that accumulation of proteins normally targeted for degradation by *UBR1* may occur in other tissues and organs affected by JBS.

Zenker M, Mayerle J, Lerch MM, et al. Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). Nat Genet. 2005;37:1345–1350.

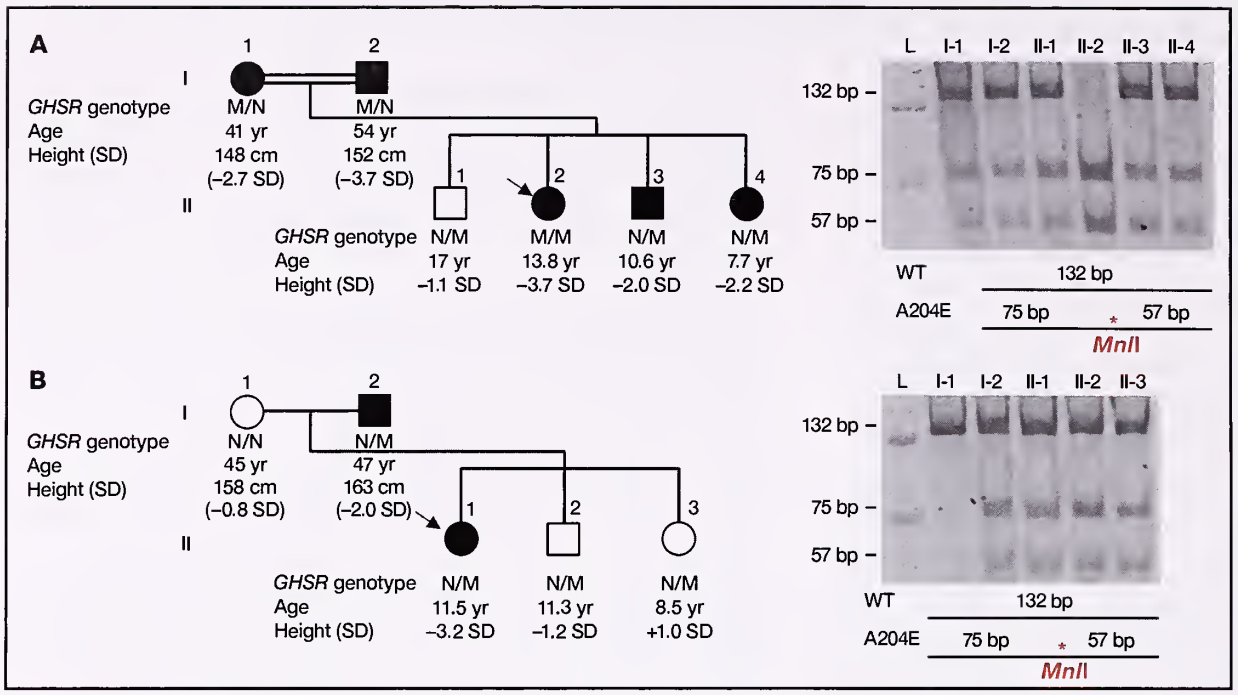
Editor's Comment: *This paper nicely documents not only the mutant gene but also the likely mechanism that accounts for the clinical features of JBS. It should be noted that the biology of ubiquitin has become much more complicated than originally suspected. For example, the number of ubiquitins added to a protein may determine its fate: many ubiquitins (polyubiquitination) usually target molecules to proteosomes, whereas addition of one or a few ubiquitins (monoubiquitination) often targets molecules to lysosomes. Monoubiquitination at multiple sites is responsible for lysosomal targeting and signal termination of many if not most activated receptor tyrosine kinases.*

William A. Horton, MD

Ghrelin Receptor Mutation: A Novel Pathogenic Mechanism of Growth Failure

In 1996, a leading article published in *Science*[1] described "a new receptor in the pituitary and hypothalamus that stimulates growth hormone release." It was cloned as the target of a family of synthetic molecules and named the growth hormone secretagogue receptor (GHSR). The endogenous ligand of this receptor is ghrelin, a hormone predominantly produced by the stomach, whose plasma levels fluctuate with food intake.[2] This hormone stimulates GH secretion and increases food intake and body weight. The G protein-coupled receptor (GPCR) displays a constitutive activity at almost 50% of its maximal capacity. The ligand-independent activity has remained unclear until the present study by Pantel and colleagues. This is the first report which identifies a GHSR missense mutation, Ala204Glu in the first exon, that segregates with short stature (SS) within 2 unrelated families, one of which also had GH deficiency (GHD).

Initially, there was a systematic search among subjects with SS leading to the identification of the same nucleotide variation in 2 unrelated patients: one with idiopathic GHD (IGHD) was found to be heterozygous for the mutation, whereas the other with idiopathic SS (ISS) was homozygous. The families of the 2 probands were analyzed. Altogether, the data showed that all individuals with SS (n=7) carried at least one mutated allele. Conversely, 3 heterozygous individuals had a normal height. The finding is in keeping with a dominant mode of inheritance and incomplete penetrance (Figure). Idiopathic GHD, diagnosed by low response to standard stimulation tests, was found in 2 cases, one in each family



Inheritance of the A204E *GHSR* mutation in families 1 and 2. (A) Family 1. (B) Family 2. Circles and squares denote female and male family members, respectively. The SD to mean height for age is given below each symbol; height values are before GH treatment. Black symbols denote a short stature. The probands are indicated by arrows. The segregation of the *GHSR* A204E allele within both families was carried out by means of a specific restriction fragment length polymorphism (the A204E mutation creates an *Mnl*I site). Reprinted with permission: Pantel J, et al. J Clin Invest. 2006;116:760-768. Copyright © American Society for Clinical Investigation. 2006. All rights reserved.

and both heterozygous. The 3 patients who received GH treatment increased their growth velocity.

The mode of action of this mutation was carefully analyzed showing that it was a significantly impaired functional activity of the receptor:

- The A204E mutant was efficiently translated into a protein, but with only a small fraction properly expressed at the cell surface. This plasma membrane fraction displayed a normal activity for ghrelin.
- The mutation led to a loss of constitutive activity of the receptor, ie, ligand-independent activity. Some experiments using an *in vitro* transcription system also suggest the absence of negative dominant effect of the mutant over the wild-type.
- Ghrelin was able to stimulate *in vitro* the c-AMP response element (CRE) pathway through the mutant receptor in spite of its decreased cell-surface expression.

Pantel et al concluded that the involvement of the *GHSR* A204E mutation in SS transmitted over 2 generations was supported by the following evidence: all patients within the 2 families carried this mutation which in turn was absent in an appropriate control population; the mutation and the amino acid polarity predicts changes in molecular activity; and the findings point to a functional importance of the GHSR constitutional activity.

The authors speculated that given the documented pharmacological effects of ghrelin on GH release, the SS results from abnormal regulation of the GH axis. The heterogeneity of the findings related to GH and insulin-like growth factor (IGF)-I in the 2 families may be related to the well-known limitations of the current methods of clinical investigation. This study, along with the mutated murine models, supports the (debated) view that ghrelin and GH/IGF-I interact in the control of growth.

Pantel J, Legendre M, Cabrol S, et al. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. J Clin Invest. 2006;116: 760–768.

Editor's Comment: *This is an elegant and well-conducted study that introduces the concept of fine regulation of growth by a mutation of a receptor which until now did not prove to be essential in achieving normal stature. In addition, it provides evidence for the in vivo importance of its ligand independent signalling as expressed by its constitutive activity. An interesting "commentary" paper is published in the same issue by Holst and Schwartz[3]; they refer to a previous German case of ISS and the same mutation[4] and also point out that obesity is an additional symptom that segregates with this mutation and a Phe279Leu mutation which shares the same effect on receptor constitutive activity. Holst and Schwartz suggest that selective loss of ghrelin receptor constitutive activity causes a syndrome of SS and obesity developing around puberty. How these molecular changes impair growth and GH secretion and furthermore, how they are involved in hunger control and obesity, remains in part speculative and challenging for future research.*

It is interesting that clinical and genetic investigation essentially by systematic structure-function analysis opens new physiological concepts. It should be taken into account when performing population studies on the multifactorial control of growth and, more specifically, by GH and IGF secretion and activity. In addition, these studies open new possible mechanisms on the weight-to-height relationship in patients with SS. The authors briefly commented on the positive response to GH therapy in 3 patients. Eventually, their data, part of which are presented in the Table, would deserve an additional publication. More studies need to be performed in order to consider the potential development of pharmacological tools in relation to this uncommon and likely pathophysiology of SS and/or obesity.

Raphaël Rappaport, MD

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Obestatin Opposes Ghrelin's Effects on Food Intake

Ghrelin, primarily a product of the oxyntic cells of the gastric fundus, is an orexigenic agent that was originally identified as the endogenous ligand of the growth hormone (GH) secretagogue receptor. Ghrelin is a 28 amino acid peptide with its serine-3 residue n-octanoylated; it is encoded by *GHRL* (OMIM 605353, chromosome 3p26-p25) and is derived from a 117 amino acid precursor peptide. Ghrelin reduces peripheral energy expenditure and enhances appetite by activating neurons that express Agouti-related peptide and neuropeptide Y that in turn inhibit expression of the anorexigenic neuromodulators that function through melanocortin and MC4R to depress appetite as well as to increase peripheral energy utilization.[1] At the carboxyl terminal of the 117 amino acid precursor, Zhang and colleagues identified an amidated 23 amino acid peptide that suppressed appetite in rats and named it "obestatin"! Its name was derived from the Latin "obedere," meaning "to devour."[2] This peptide decreased food intake whether administered peripherally or centrally, suppressed body weight gain, delayed gastric emptying and inhibited jejunal contractility. The investigators next identified the putative receptor for obestatin–the orphan G-protein-coupled receptor (GPR)-39–that functioned by enhancing adenylyl cyclase activity (gαs). Although derived from the same precursor, the secretory patterns of ghrelin and obestatin differed significantly. In response to fasting, serum concentrations of immunoreactive ghrelin increased while those of obestatin did not change. The investigators concluded that the physiological role of obestatin in the regulation of energy consumption and use had yet to be determined.

Zhang JV, Ren P-G, Avsian-Kretchmer O, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science. 2005;310:996–999.

Editor's Comment: *That one gene can encode more than one peptide product is well illustrated by POMC whose primary product proopiomelanocortin is the precursor peptide from which ACTH, MSH, and β-endorphin are derived. Similarly, CALCA encodes calcitonin and calcitonin gene-related peptide. However, it appears unique that one peptide gives rise to products that apparently antagonize each other's actions and yet are differentially secreted (or alternatively catabolized). One eagerly awaits elucidation of the regulation of obestatin secretion and its physiologic role. Although obestatin did not stimulate or suppress the secretion of growth hormone from cultured rat pituitary cells in vitro, its effects on ghrelin-stimulated growth hormone release were not examined. It would be of interest if it had properties similar to those of somatostatin in this regard. Study of the effect of obestatin on ghrelin-mediated food intake would also be of immense interest.*

Allen W. Root, MD

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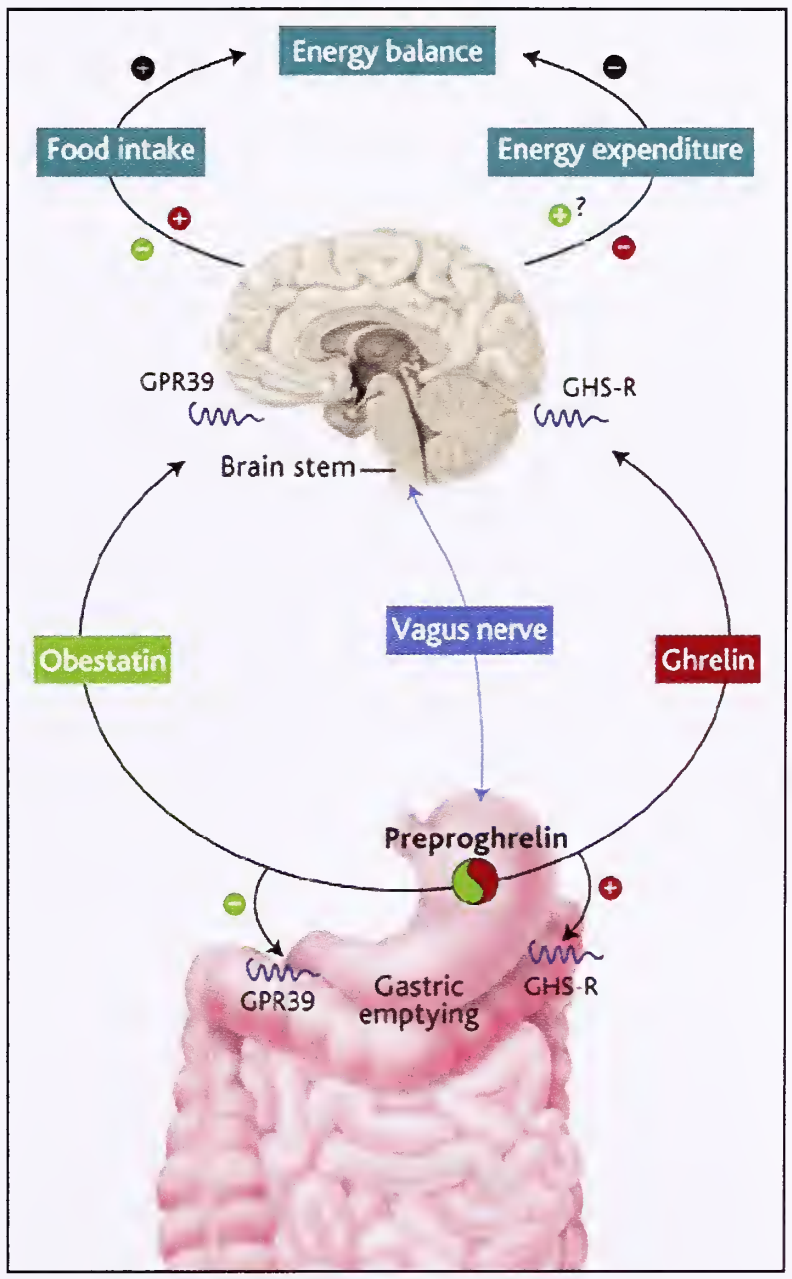

The Yin and Yang Personalities of Ghrelin and Obestatin. Both hormones derive from the same precursor protein and are predominantly secreted by the stomach and released into the blood. Each acts on a different receptor (GPR39 and GHS-R, as shown) and has an opposite effect on food intake, body weight, and gastrointestinal motility.
Reprinted with permission. Nogueiras R, Tschop M. Science. 2005;310:985. Copyright ©AAAS, 2005. All rights reserved. PHOTO CREDIT: K. SUTLIFF/SCIENCE

Endocrinological and Auxological Abnormalities in Children with Optic Nerve Hypoplasia: A Prospective Study

Ahmad and associates performed a prospective observational study of 47 children with optic nerve hypoplasia (ONH [deMorsier's syndrome]) who presented to the Pediatric Ophthalmology clinic at Children's Hospital Los Angeles. Subjects 3 years of age and under were enrolled in the study and were followed annually until 5 years of age for visual growth and neurodevelopment outcomes. Although 170 subjects have been enrolled, the data presented are for the first 47 subjects to have completed the study. All subjects had baseline endocrinological, electrophysiological, and neuroradiological findings. Growth hormone (GH) status was defined by insulin-like growth factor (IGF)-I and/or IGF binding protein (BP)-3 or subnormal GH responses to glucagon stimulation. Height (or length) and weight were measured at each visit.

Hormonal dysfunction was found in 71.7% of these children. A growth hormone axis abnormality was observed in 64.1%, hyperprolactinemia in 48.5%, hypothyroidism in 34.9%, adrenal insufficiency in 17.1%, and diabetes insipidus in 4.3%. There was no association between endocrine abnormalities and unilateral versus bilateral ONH. In addition, the absence of the septum pellucidum or other pituitary abnormalities was not associated with endocrinologic function. There was no statistically significant difference in the median start versus end height SDS, but there was a significant increase noted for the median weight SDS. In the cohort, 44.4% were >85th percentile for weight at the end of the study. There were 27 subjects who had both IGF-I and IGFBP-3 assessed. The data were dichotomized as "both normal" or "at least one abnormal hormone surrogate." Using this division, there was no significant difference in the median change in height, weight, or in body mass index (BMI) over time. Eight of the subjects received GH replacement. Of the 19 subjects not receiving GH therapy, 10 had one abnormal GH surrogate. Although the change in height was statistically significant for those receiving GH therapy, those children who did not receive GH treatment continued to grow, with significant BMI increase.

The authors pointed out that there is an unclear understanding of the etiology of ONH. The current study which confirmed a high prevalence of endocrinopathy showed no association between endocrine abnormalities and unilateral versus bilateral ONH, although subjects with a pituitary abnormality on neuroimaging had an endocrinopathy. Seventy-one percent of those with a normal pituitary gland also had an endocrinopathy. The authors speculated that one of the possibilities for explaining the weight gain might be a decreased lipolytic activity resulting from the absence of GH, as suggested in patients with Prader-Willi syndrome.

Ahmad T, Garcia-Filion P, Borchert M, Kaufman F, Burkett L, Geffner M. Endocrinological and auxological abnormalities in young children with optic nerve hypoplasia: a prospective study. J Pediatr. 2006;148:78–84.

Editor's Comment: *This is a very interesting observational study, which provides important information for pediatric endocrinologists, geneticists, and pediatricians who care for children with ONH. Importantly, it demonstrates that it is not sufficient to evaluate these children endocrinologically at only one point in time. In addition, it is not sufficient to assume that these children do not have GH deficiency because they continue to experience linear growth. Indeed, the authors have shown that many of these children continue to grow linearly and to gain excessive weight. The suggestion that these children may be candidates for GH treatment regardless of their GH surrogate status is appealing and deserves further investigation.*

William L. Clarke, MD

Growth Hormone Receptor Exon-3 and Response to Growth Hormone Treatment

A polymorphism in the growth hormone receptor (GHR) gene, the presence or absence of exon-3, has recently been shown to influence the 1- and 2-year growth response to recombinant human growth hormone (rhGH) therapy in children without GH deficiency (GHD). To study the influence of GHR-exon-3 genotype on the short- and long-term response to rhGH therapy in children with GHD, Jorge et al genotyped and followed the first year growth velocity following rhGH treatment in 58 children (36 boys, 22 girls) who remained prepubertal and the adult height of 44 patients (included 27 patients analyzed for the first-year response) after 7.5 ± 3.0 years of treatment.

Clinical and laboratory data at the start of treatment were indistinguishable among patients carrying *GHR*-exon-3 genotypes. Patients carrying at least one exon-3 deleted *GHR* (*GHR*d3) allele had a significantly better growth velocity in the first year of treatment (12.3 ± 2.6 vs 10.6 ± 2.3 cm/year, $p<0.05$) and achieved a taller adult height (final height SDS of –0.8 ± 1.1 vs –1.7 ± 1.2, $p<0.05$) when compared with patients homozygous for *GHR* full-length alleles (*GHR*fl). They conclude that patients with GHD who are homozygous for *GHR* exon 3fl were less responsive to short- and long-term rhGH therapy. Approximately half of the population is homozygous for *GHR*fl; thus, future studies adjusting rhGH therapy to genotype may improve outcome to therapy.

Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ. Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab. 2006;9:1076–1080.

Editor's Comment: *Different variables can influence the growth velocity and the final height of children treated with rhGH, but so far there is no way of accurately predicting response to therapy. Duration of treatment, height SDS at the start of treatment, bone age delay, midparental height, and growth velocity during the first year of treatment, are some of the variables which could influence final height after therapy. However, as suggested by Jorge et al, these variables only partially explain the inter-individual variability response to rhGH treatment in children with GHD. The GHR gene is an obvious candidate to influence the response to rhGH. The GHR gene is located in the short arm of chromosome 5; two of the most common isoforms of GHR in humans are generated by retention of GHRfl or exclusion of GHRd3. The frequency of each allele in humans ranges from 68% to 75% for GHRfl and from 25% to 32% for GHRd3.*

Patients reported in this paper with GHD who were homozygous for GHR exon 3fl were less responsive to short-and long-term rhGH therapy. However, Pilotta et al[1] recently evaluated 54 GHD children treated for at least one year with rhGH; they found no significant differences in growth velocities between groups of subjects defined by polymorphic genotypes, and concluded that the most common polymorphisms, alone or in association, did not appear to affect the growth response to rhGH in GHD children. On the other hand, studies by Dos Santos et al[2] and Binder et al[3] support the theory that there is increased responsiveness to high dose rhGH in association with GHRd3 genotype in patients with Turner syndrome, small for gestational age (SGA), and idiopathic short stature; the magnitude of this effect may depend on the primary cause of the short stature. The Binder group demonstrated that girls with Turner syndrome who were homozygote for the GHRd3 variant showed the highest increment in height velocity and exceeded their growth prediction, whereas short children born SGA demonstrated only a mildly increased response to high-dose rhGH in the presence of the GHRd3 variant. Genotyping of the GHRd3 protein polymorphism may prove to be a tool for a more precise understanding of rhGH effects on growth and for the individualization of rhGH dosing in both GHD and non-GHD children; however, its effectiveness is still in doubt.

Roberto Lanes, MD

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IGF-I and IQ in Middle Childhood

Gunnell et al examined the association between circulating levels of insulin-like growth factor (IGF)-I, its main binding protein, IGFBP-3, and subsequent measures of IQ. Data were obtained from the Avon Longitudinal Study of Parents and Children (ALSPC, n=13 617). The study consisted of 547 white singleton children (301 boys, 246 girls), with IGF-I and IGFBP-3 measurements obtained at a mean age of 8 years and IQ measured with the Wechsler Intelligence Scale for Children (WISC-III) at a mean age of 8.7 years. Speech and language were also measured by the Wechsler Objective Reading Dimensions (WORD; assessed at 8.7 years) and Wechsler Objective Language Dimensions (WOLD; assessed at 7.5 years) tests. Some children (n=407) had IGF-I levels measured at approximately 5 yeas of age in a previous study.

The mean IGF-I (ng/mL) level at age 8 years was 142.6 (± 53.9) and 154.4 (± 51.6) for boys and girls, respectively. For every 100 ng/mL increase in IGF-I, IQ increased by 3.18 points (p=.019) for boys and girls combined. This relationship achieved statistical significance only for girls. A statistically significant association was not detected between IGFBP-3 or IGF-I/IGFBP-3 ratios and IQ. WISC-III subtests are classified as Verbal or Performance: associations between IGF-I and IQ were restricted to the Verbal component. The IGF-I levels were not significantly associated with either WOLD or WORD test scores for the combined sample of boys and girls. A positive statistically significant association between IGF-I levels and WORD scores was detected for girls, but not for boys. Associations between IGF-I levels at age 5 and WISC-III scores were similar to those for IGF-I levels measured at age 7 to 8, applied to both the boys and girls, but were restricted to the Verbal IQ.

Follow-up analyses were performed statistically, controlling for potential confounding variables. Introducing birth weight (adjusted for gestation), breastfeeding, and BMI to the regression model strengthened the association between IGF-I and IQ; whereas controlling for maternal education and IGFBP-3 attenuated the association, as did adjustment for housing status and family socioeconomic status. The authors suggest that rather than confounding the associations of IGF-I levels with IQ, parental education and socioeconomic status may serve as markers of their offspring's intelligence. The authors concluded "Offspring IGF-I levels are likely to be associated with parental IGF-I levels, through shared genetic influences. This study provides some preliminary evidence that IGF-I is associated with brain development in childhood. Additional

longitudinal research is required to clarify the role of IGF-I in neurodevelopment. Because IGF-I levels are modifiable through diet and other environmental exposures, this may be one pathway through which the childhood environment may influence neurodevelopment."

Gunnell D, Miller LL, Rogers I, Holly JMP, the ALSPAC Study Team. Association of insulin-like growth factor I and insulin-like growth factor-binding protein-3 with intelligence quotient among 8- to 9-year-old children in the Avon Longitudinal Study of parents and children. Pediatrics. 2005;116:e681–e686.

Editor's Comment: *The prospect of an association between IGF-I, brain development, and intelligence is not new,[1] but remains intriguing. The importance of the Gunnell study lies in the cohort design of the ALSPC, the quality of the psychological/cognitive assessments, and detailed characterization of important contextual variables in child development (eg, diet and socioeconomic status of the family). Evidence that growth factors (rather than psychosocial stress associated with short stature) may be responsible for educational and vocational outcomes suggests that stature and growth can be viewed as proxies for other biologic events rather than as a focus for its own sake.*

Findings from a controlled study by Kranzler and colleagues[2] on the intellectual ability of children with growth hormone receptor deficiency (GHRD) (and accompanying severe IGF-I deficiency) are difficult to reconcile with the Gunnell report. Kranzler compared the intellectual ability of 18 school-age Ecuadorian GHRD probands with that of 42 relatives and 28 controls. The intellectual ability of those with GHRD was not significantly different from their relatives, and was comparable to controls. Furthermore, homozygosity or heterozygosity for the mutation in the GHR gene common to Ecuadorian patients was unrelated to intelligence. The authors concluded that GH-induced IGF-I production is not required for normal brain growth in utero or for postnatal intellectual development.

It may be overly simplistic to question, but if circulating values of IGF-I are positively related to intellectual function, then would GH-mediated increases in IGF-I result in higher performance? Indirect supportive evidence comes from a study of the effects on IQ scores of GH administered to children born small for gestational age.[3] Growth hormone treatment was associated with significant increases in relative height along with improved IQ. Because it can be assumed that GH treatment raised IGF-I levels, then perhaps IGF-I effects on the central nervous system mediated the effects of GH on intellectual ability. Interestingly, it was only the Performance IQ that showed improvement with GH treatment; the opposite pattern was observed in the Gunnell study. Clearly, all these findings require replication, and hopefully future investigations will be guided by a priori predictions regarding the effects of growth factors on brain development and function in order to reduce the probability of Type I errors.

David E. Sandberg, PhD

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In This Issue
Reviews & Comments

E-Reviews & Comments

THE SOTOS SYNDROME - *NSD1* HAPLOINSUFFICIENCY: CEREBRAL GIGANTISM UPDATE

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INTRODUCTION

Cerebral gigantism (OMIM 117550) is characterized by excessive pre- and postnatal growth, a characteristic face, and developmental delay with a prevalence of ~1:14,000 births.[1] In more than 90% of patients, Sotos syndrome is due to haploinsufficiency of *NSD1* (***N***uclear Receptor-Binding ***S***u-var, Enhancer of Zeste, and Trithorax ***D***omain Protein ***1***, chromosome 5q35, OMIM 606681).[2] Cerebral gigantism is thus a genomic disorder–a pathologic state due to loss, gain, or disruption of a dosage-sensitive gene that results in a recognized phenotype.[3]

CLINICAL CHARACTERISTICS

In patients with cerebral gigantism, rapid linear growth begins during gestation; at birth, length is more than +2 standard deviations (SD) above mean length for gestational age and gender in 85% of neonates, while birth weights are usually within the high normal range. Neonates with Sotos syndrome may also have prolonged jaundice, hypotonia, and feeding difficulties.[2] Linear growth remains rapid throughout infancy and childhood. Because skeletal maturation is also advanced, adult heights of patients with Sotos syndrome are usually near or slightly

From The Editor's Desk

Dear Colleague:

This column usually highlights the content of the journal; in this issue I want to bring to your attention the e-reviews and editorial comments. This section was expanded to 10 reviews of current articles as more of our readers are taking advantage of this feature. The online reviews and comments are often slightly longer or contain more detail than the printed reviews. The article on sex assignment attitudes of pediatric urologists is interesting and worthy of your consideration. The new diagnostic imaging techniques in congenital hypothyroidism should become available in all medical centers. The issues of quality of life and mental health of adolescents seeking bariatric surgery need be considered as we now deal with this problem on a frequent basis. Not least are the important data discussed in the other reviews dealing with clinical conditions such as CAH, Klinefelter's syndrome, and progeria, as well as reviews of experimental data on GHR, longevity and calorie restriction, hippocampal GH, and congenital contractures.

The lead article is a much-needed update on Sotos syndrome which was first described in 1964, at a time when heterozygous microdeletions in *NSD1* were not known, but are present in over 90% of these patients. Drs. Root and Diamond reviewed the genetic considerations of this condition and didactically clarified the mechanisms of the disease. The 8 reviews and comments complete this issue with an array of the most pertinent recent advances in the field.

Other pertinent additions to www.GGHjournal.com include new sections on clinical guidelines and clinical trials. These are most useful for those who want current recommendations or to apprise themselves of the clinical research trials ongoing in the field and other areas. The search capability and archives sections have been enhanced and a very important feature added—a CME offering is now available on the resources webpage.

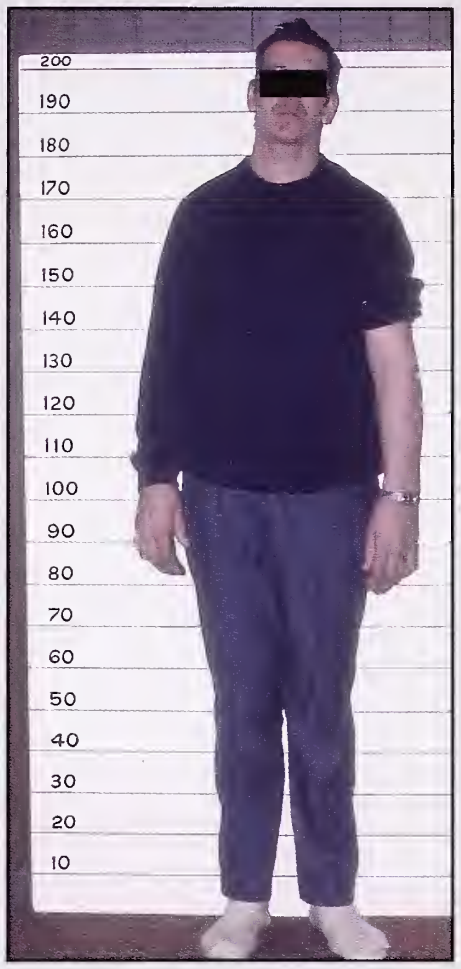
Fima Lifshitz, MD

Growth, Genetics & Hormones is supported by an unrestricted educational grant from

above +2 SD; however, adult stature usually exceeds target height by an average of 11 cm in males and 6 cm in females (Figure 1).

Occipito-frontal head circumference is also increased during infancy and childhood and remains above the 97th percentile in most adults with Sotos syndrome. However, in 10% of subjects with cerebral gigantism, height and head circumference remain within the normal ranges.[4] The "acromegalic-like" face of the patient with cerebral gigantism is characterized by a high, broad, and bossed forehead with sparse fronto-temporal hair, long and narrow face, down-slanting of the palpebral fissures, malar flushing, and a sharply pointed, prognathic mandible that becomes more evident over time.[5,6] The palate is highly arched; hands and feet are prominent; and scoliosis occurs frequently. Subjects with Sotos syndrome also have anomalies of the cardiovascular (patent ductus arteriosus, atrial septal defect), genitourinary (agenesis, duplication, vesicoureteral reflux, hypospadias, cryptorchidism), and central nervous systems (hypoplasia of the corpus callosum, ventricular dilatation, enlarged extracerebral fluid spaces); electroencephalographic abnormalities and seizures occur in some subjects. Most but not all patients with cerebral gigantism have mild to severe developmental delay, a problem that may ameliorate somewhat as the patient ages.[7] In addition, Sotos syndrome patients may develop aggressive behavior and may manifest psychoses in adulthood.[8,9] Neoplasms develop in 2% to 4% of children with cerebral gigantism, including acute lymphoblastic leukemia, T-cell lymphoma, Wilms tumor, sacrococcygeal teratoma, presacral ganglioneuroma, hepatocellular carcinoma, neuroblastoma, and ganglioglioma.[10,11]

Figure 1.



A 22 year old male with cerebral gigantism.

PATHOGENESIS

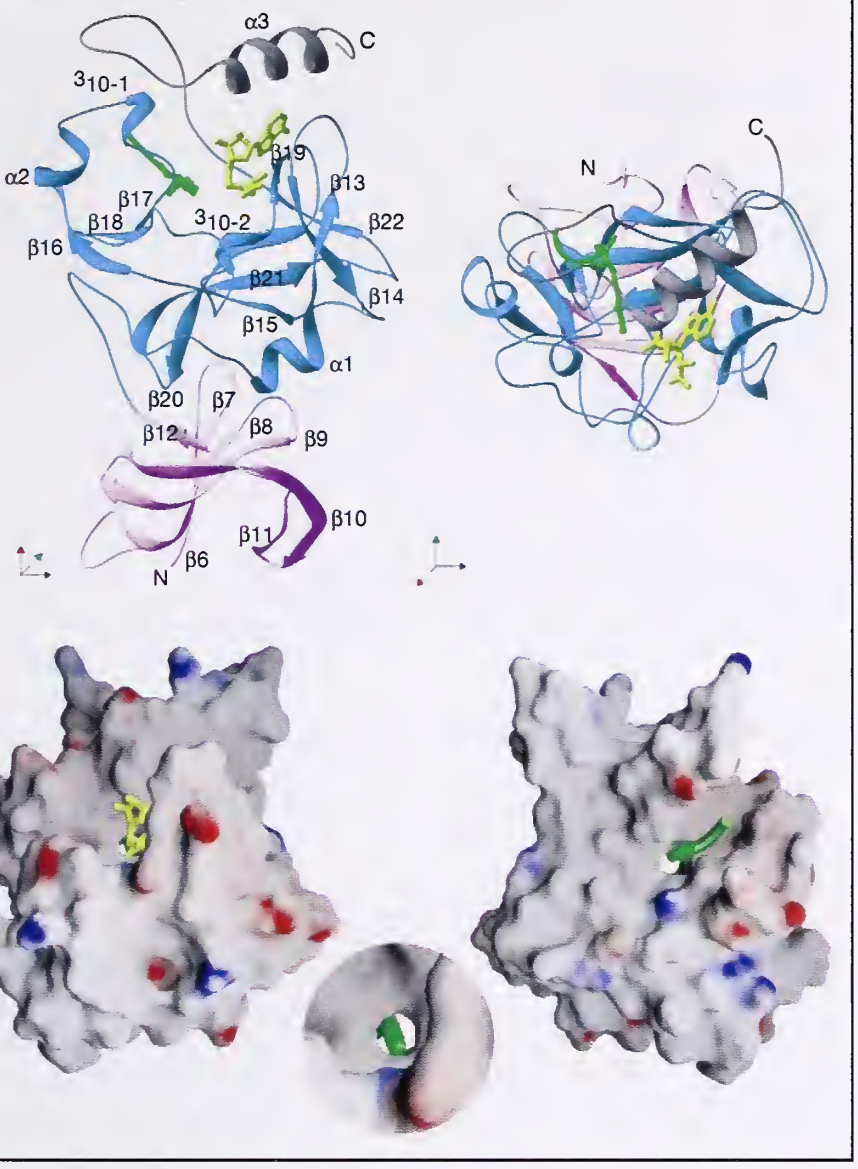
The mechanism(s) underlying the extreme growth of patients with Sotos syndrome is unknown. Growth hormone secretion is normal in patients with cerebral gigantism; serum concentrations of insulin-like growth factor (IGF)-I and acid labile subunit have been normal, a bit low, or somewhat elevated in various reports.[12] In some subjects, serum levels of IGF-II and IGF-binding proteins (IGFBP)-3 and -4 have been within the low normal range. The rate of IGFBP-3 proteolysis was accelerated in one report, suggesting that free IGF-I values might be increased in this disorder.[12] Prostate specific antigen (PSA) is one of several proteolytic enzymes that degrade IGFBP-3.[13,14] Perhaps measurements of PSA levels in patients with cerebral gigantism would be of interest and contribute to our understanding of the pathogenesis of this disorder.

GENETICS

Heterozygous microdeletions and loss-of-function mutations in *NSD1* resulting in haploinsufficiency of the gene product have been identified in more than 90% of patients with Sotos syndrome.[2,4,5] *NSD1* contains 23 exons and encodes a 2596 to 2696 amino acid, broadly expressed protein (brain, muscle, kidney, spleen, thymus, lymph node, lung) that functions as a co-regulator of transcription by interacting with nuclear transcription factors and as a histone methyltransferase.[11] Within the structure of *NSD1*, there is a SET domain, a conserved sequence of approximately 150 amino acids that remodels chromatin structure by histone methylation, thereby modulating gene transcription; *NSD1* specifically methylates lysine-36 of histone H3 and lysine-20 of histone-H4.[15] By inserting its side chain into a cleft within the SET-containing protein, the selected histone H3/4-lysine accesses both the enzymatic site and its methyl donor S-adenosyl-L-methionine.[16] Figure 2 shows a structure of the SET 7/9 ternary complex. This structure is highly specific for the histone methyltransferase target. Methylation of one or the other histone H3/4-lysine residues usually, but not necessarily always, exerts an inhibitory (silencing) effect on the transcription of a targeted gene. Encoded within *NSD1* are several additional functional domains, including one SET-associated cysteine-rich domain, two nuclear receptor interactive domains (exon 2), two proline-tryptophan-tryptophan-proline (PWWP) domains (exons 3-4, 15-17), and 5 plant homeodomains (PHD) (exons 11-17, 22).[5,6] A PHD has a zinc-finger structure that permits interaction with chromatin, while the PWWP domains are involved in protein-protein interaction.

NSD1 binds to transcription factors and co-factors where it may behave as either a co-activator or co-repressor, depending on which of its two nuclear interactive domains is involved.[5,11] By binding to the intact or carboxyl terminal region of the nuclear androgen receptor (AR) through its activating nuclear receptor interactive domain, *NSD1* acts as a co-regulatory factor that enhances AR transcriptional

Figure 2.



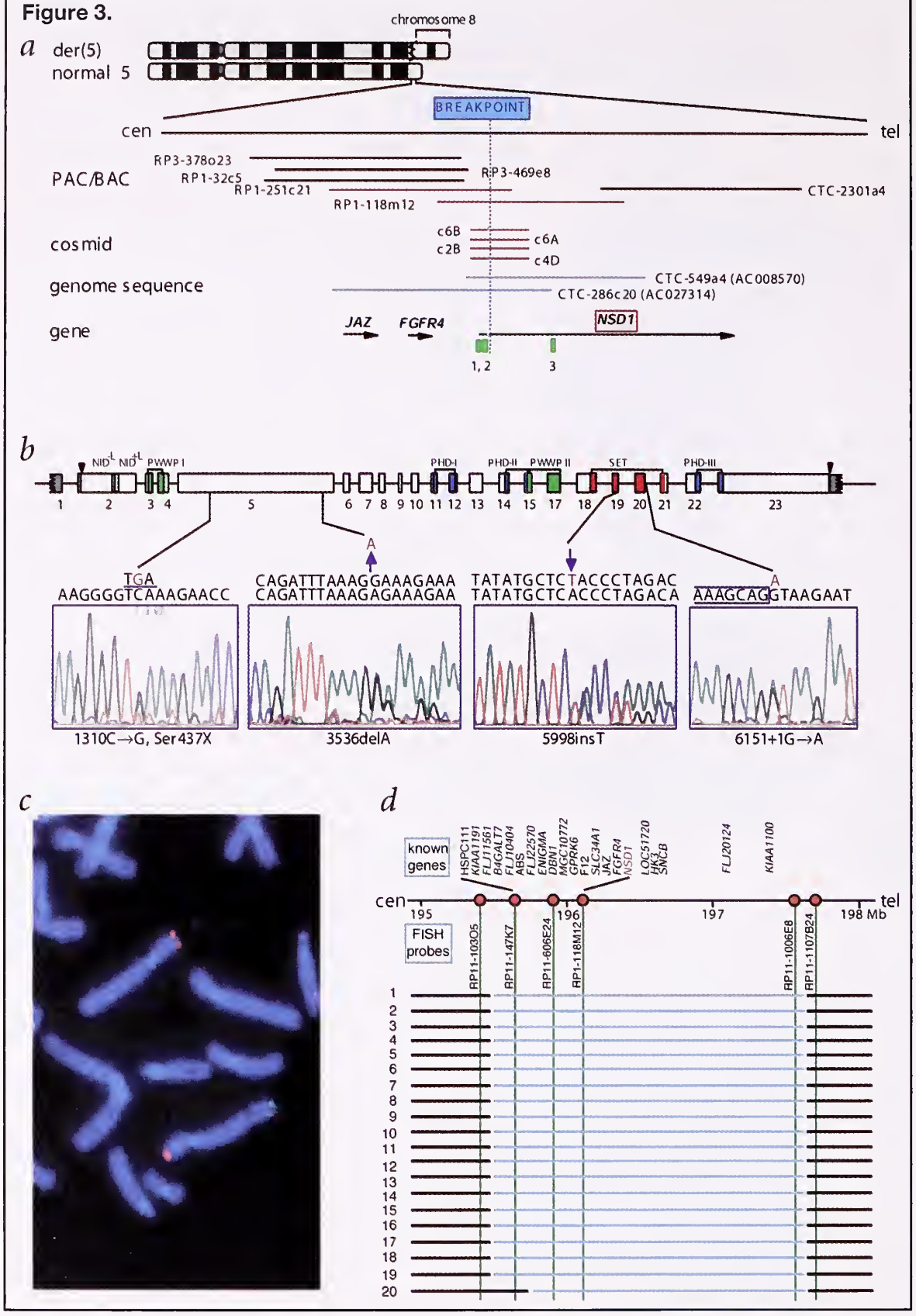
Structure of the SET 7/9 ternary complex. **a,** Two orthogonal views of the SET 7/9 ternary complex in ribbons representation. The N-terminal domain is colored pink, the SET domain is blue and the C-terminal segment is grey. The H3 peptide is indicated in green, with the side chain of methylated Lys 4 shown. The S-adenosyl-L-homocysteine (AdoHcy) cofactor is colored yellow. The secondary structure elements are labeled according to our earlier structure. Two small turns of the 310 helix are also labeled. **b,** Two views of the SET domain are shown in a surface representation colored according to electrostatic potential (the two views are related by a twofold rotation about a vertical axis). The left panel shows AdoHcy colored yellow; the right panel shows the H3 peptide colored green. The inset panel shows a close-up view of the lysine access channel containing the methyl lysine side chain as viewed from the S-adenosyl-L-methionine (AdoMet)-binding site.

activity. *NSD1* also interacts with NIZP1, a zinc-finger DNA-binding repressor of transcription that directs the histone methyltransferase SET domain of NSD1 to targeted gene promoters.[17] *NSD1* and other SET-domain containing proteins interact with factors that regulate cell growth and have been implicated in several human malignancies such as acute myeloid leukemia of childhood.[18] However, the mechanism(s) by which inactivating mutations of *NSD1* lead to the clinical manifestations of Sotos syndrome remains unclear at present. Since homozygous *NSD1*$^{-/-}$ mutant mouse embryos succumb very early in gestation, *NSD1* is also crucial for early post-implantation mammalian fetal development.[13] However, the *NSD1*$^{+/-}$ mutant mouse is phenotypically normal.

Microdeletions of 1.9Mb-encompassing *NSD1* are the most common mutations identified in subjects with cerebral gigantism of Japanese ancestry[19] (Figure 3). Mechanistically important in the process that leads to microdeletions of *NSD1* in this population is non-allelic homologous recombination or unequal rearrangement of low-copy repeat sequences that flank the distal and proximal breakpoints that encompass *NSD1*.[4,20,21] Preferentially, microdeletions of *NSD1* are of paternal origin in Japanese patients with Sotos syndrome. Their fathers have a heterozygous inversion of nucleotides flanking *NSD1* on chromosome 5 that predisposes to unequal intrachromosomal recombination during meiosis that leads to microdeletions (or duplications) in their progeny.[11,21,22] In western populations, microdeletions of *NSD1* are variable in size, due to interchromosomal rearrangement, and far less frequent, accounting for less than 10% of the identified *NSD1* mutations in patients with Sotos syndrome. More than 100 intragenic inactivating splice site, frameshift (due to small insertions and deletions), nonsense, and missense *NSD1* mutations that account for more than 90% of the genetic abnormalities identified in non-Japanese patients with cerebral gigantism have been identified.[5,23] Missense mutations are clustered between exons 13 and 23 within conserved functional domains.[24]

There is a vague phenotype-genotype relationship in Sotos syndrome: thus, macrosomia, developmental delay, and minor anomalies are present in these patients with either intragenic mutations or gene microdeletions. However, patients with cerebral gigantism and microdeletions of *NSD1* tend to have rather severe developmental delay and major structural anomalies of the central nervous, cardiovascular, and genitourinary systems, but only modest overgrowth.[4,25] On the other hand, patients with intragenic mutations may express less severe anomalies but demonstrate greater linear overgrowth.[2,25] Nevertheless, unrelated Sotos syndrome patients with identical mutations in *NSD1* may have different phenotypes.[4] In neonates with mutations in *NSD1*, birth length is substantially greater than in subjects with clinical Sotos syndrome but an intact gene.[26] Arm span relative to height and hand length relative to age are substantially greater in patients with Sotos syndrome (due to a mutation in *NSD1*) than in subjects with clinical

Figure 3.



NSD1 mutations in individuals with Sotos syndrome. ***a,*** BAC/PAC/cosmid map spanning the 5q35 breakpoint. Red and blue horizontal lines indicate clones spanning the breakpoint (detected by FISH analysis) and complete genomic sequences, respectively. Arrows indicate genes, and green boxes below *NSD1* represent exons 1, 2 and 3. ***b,*** Genomic structure of *NSD1* and four point mutations found in individuals with Sotos syndrome. Open and gray boxes and arrowheads indicate exons, the 5' and 3' untranslated regions, and start and stop codons, respectively. Specific domains are indicated by colored boxes, and sequence traces disclose mutations in lower row. ***c,*** FISH analysis of the affected individual harboring the deletion. Absence of a FISH signal for RP1-118m12 containing *NSD1* (green) along with the presence of 5pter signals (red) on the individual's chromosome 5 is apparent. ***d,*** Summary of FISH deletions in 20 affected individuals. Known genes, probes used and their genomic locations are indicated in the upper row. Numbers (1–20) and black and blue lines represent affected individuals, regions without deletion and those regions deleted, respectively.

manifestations of cerebral gigantism and normal *NSD1*.[27] Developmental delay may be more severe in patients with a mutation in *NSD1* than in those with clinically diagnosed cerebral gigantism.

Mutations or deletions of *NSD1* most often arise *de novo* and thus the risk of familial recurrence to phenotypically and genotypically normal parents is low. Nevertheless, a patient with Sotos syndrome due to an intragenic mutation has a 50% risk of transmitting this mutated gene to an offspring.[7] No instance of germline *NSD1* mosaicism has been observed to date. Intragenic mutations in *NSD1* have also been reported in patients with Weaver syndrome (OMIM 277590), an overgrowth disorder that is characterized by macrocrania, hypertelorism, large ears, retrognathia, hypotonia, developmental delay, loose skin folds, dysplastic deeply set nails, sparse hair, and hoarse cry as well as various skeletal anomalies.[24] Patients with Weaver syndrome very rarely develop tumors. In 2 out of 52 patients with the Beckwith-Wiedemann syndrome (BWS - OMIM 130650) who presented with *in utero* macrosomia, macroglossia, hemihyperplasia, and abdominal wall defects, mutations in *NSD1* were also reported.[28] A microdeletion of *NSD1* was detected in an infant girl with features of both Sotos and Nevo syndromes (OMIM 601451).[29] However, there is evidence that suggests that the Weaver, BWS, and Nevo syndrome patients studied in these reports were more likely to have had clinical variations of Sotos syndrome.[6,30] Anomalies of 11p15, the site of imprinting errors associated

Table 1. Scoring System for Clinical Diagnosis of Cerebral Gigantism.

Criteria		Score
Facial characteristics	5 or 6 present	5
	2 to 4 present	3
	≤1 present	0
Growth	Height SDS - TH SDS >2	2
	Height SDS - TH SDS ≤2 (past data)	1
	Height SDS - TH SDS ≤2	0
Head circumference	≥2 SDS	1
	<2 SDS	0
Development	IQ <90 – delayed	1
	IQ ≥90	0
Bone age	Consistently advanced	2
	Adult	1
	Normal for age	0

Sum 9-11: Typical Sotos syndrome

Adapted from reference 12.
Facial characteristics: frontal bossing, high hairline, dolicocephaly, prominent chin, highly arched palate, anti-mongoloid slant of palpebral fissures
Growth - all measurements before adult height
SDS - Standard deviation score
TH - Target height

Table 2. Recommended Surveillance of Children With Cerebral Gigantism for Tumor Development: Yearly Frequency.

Age	Physical examination	CBC	Abdominal ultrasound	Chest x-ray	α-Feto-protein	β-hCG	Urine catecholamines
Birth to 4 years	4	3	2	1	2	2	1
4 to 10 years	3	3	2	1	2	2	1

Adapted from reference 10.

with BWS, were identified in 2 out of 20 patients with cerebral gigantism, including one with paternal isodisomy of 11p15 of the *H19* locus and one with partial isolated demethylation of *KCNQ1OT*, perhaps suggesting a functional relationship between *NSD1* and the imprinting centers on 11p15.[2,28]

DIAGNOSIS AND MANAGEMENT

The diagnosis of Sotos syndrome is established by clinical findings (characteristic facial features, prenatal and postnatal overgrowth, persistently enlarged head circumference, developmental delay, and advanced bone age [Table 1]) and confirmed by identification of a mutation in *NSD1*. Since this disorder is genetically heterogeneous, absence of a mutation does not negate the diagnosis; an abnormal methylation pattern on chromosome 11p15 might be investigated in patients with intact *NSD1*.[28] Cerebral gigantism is to be differentiated from Weaver syndrome and other overgrowth syndromes both on clinical grounds and by the presence of mutations in *NSD1*. Treatment is symptomatic and focuses on monitoring of growth and periodic surveillance for associated systemic anomalies or development of neoplasms and on factors that may ameliorate developmental delay and behavioral problems. It has been recommended that children with Sotos syndrome be surveyed frequently for tumor development through 10 years of age. Complete physical examinations and blood counts should be done 3 to 4 times each year; abdominal ultrasound studies, α-fetoprotein, and β-hCG measurements twice yearly; and chest x-ray and urine catecholamine determinations once each year (Table 2).[10] Given the relatively low incidence of tumors in these patients, these recommendations may be excessive.[11]

CONCLUSION

Cerebral gigantism is an overgrowth syndrome characterized by increased *in utero* and postnatal growth, an adult height that is within or slightly above the upper normal range, macrocephaly, a characteristic face, and variable degrees of developmental delay. There is a high incidence of cardiovascular, central nervous and genitourinary malformations in these patients. Tumors occur in 2% to 4% of patients with cerebral gigantism, usually before 8 to 10 years of age. Mutations in *NSD1* seem to be quite specific for Sotos syndrome.[6] Indeed, all subjects with documented mutations in *NSD1* have some manifestation(s) of cerebral gigantism. The majority of mutations in *NSD1* have occurred *de novo*, but the risk for development of Sotos syndrome in the offspring of a patient with a mutation in *NSD1* is 50%. Periodic surveillance for tumor development is recommended in children with Sotos syndrome.

Resources

Sequence analysis of *NSD1* may be obtained through The Greenwood Genetics Center, Greenwood, SC.
The Sotos Syndrome Support association may be contacted at www.well.com/user/sssa.

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REVIEWS & COMMENTS FROM THE LITERATURE

Autoimmune Growth Hormone Deficiency: Whittling Away at Some of the Idiopathics

Lymphocytic hypophysitis is a recognized cause of growth hormone deficiency (GHD) in adults, either isolated or associated with deficiency of other pituitary hormones. Histopathological diagnosis on pituitary biopsy is considered the gold-standard test, though antipituitary antibodies (APA) have been recently identified in adults with idiopathic GHD (IGHD) or GHD and other autoimmune endocrinopathies. The APA-positive adults with IGHD all had documented childhood-onset GHD; thus the authors aimed to investigate the presence of APA in prepubertal children.

De Bellis and colleagues studied APA in 3 groups of prepubertal patients: 26 with IGHD, 60 with idiopathic short stature (ISS), and 33 with organic GHD (destructive lesions or developmental malformations of the hypothalamus-pituitary). The definition of IGHD was a height z score below –2 SD, growth velocity <25th centile, delayed skeletal development, and blunted GH response (<10 µg/L) on both arginine and insulin stimulation tests; all patients had GH peaks <5 µg/L and abnormally low IGF-I levels for age and gender. The definition of ISS was the same except that GH peaked at >10 µg/L on at least one stimulation test. Ten of the 60 ISS patients had abnormally low IGF-I levels. MRI was normal, as were all other pituitary hormone functions, in both groups. Sera from 40 age- and sex-matched normal children were collected as controls. The APA were detected by indirect immunofluorescence on cryostat sections of young baboon pituitary, with fluorescein isothiocyanate (FITC)-conjugated goat anti-human immunoglobulins; positive samples were defined as titers >1:8. For sera that were APA positive, antibody specificity for the different anterior pituitary cell types was determined by a 4-layer double immunofluorescence technique, co-localizing the FITC-conjugated anti-human IgG antibody with rodhamine-conjugated antibodies directed against each of the pituitary hormones.

The APA antibodies were positive in 27% of the children with IGHD and 23% of those with ISS, but were negative in those with organic GHD and in normal controls. The APA titers ranged from 1:32 to 1:128 in the former and 1:16 to 1:64 in the latter group. Immunostaining confirmed selectivity for pituitary somatotrophs with minor staining of lactotrophs but no other cell type. Three of the 7 APA-positive GHD patients and 8 of the 14 ISS patients had parents or first degree relatives with autoimmune endocrinopathy or non-endocrine disease. Within the ISS group, all 10 patients with abnormally low IGF-I levels were APA positive.

Nineteen of the 60 patients with ISS were re-evaluated 2 years later; 11 had originally been APA negative and 8 APA positive. All 11 negative patients remained APA negative, retained normal GH response to provocative testing, and normal age-dependent increase in IGF-I levels. In contrast, all 8 APA-positive patients demonstrated an increase in their autoimmunity, with titers ranging from 1:32 to 1:128. IGF-I levels remained abnormally low in all 8 patients. Furthermore, 7 developed failure on provoked GH testing. MRI was normal.

The authors concluded that APA against somatotrophs are present in 27% of children with IGHD and 22% of children with ISS. The APA may therefore indicate autoimmune hypophysitis despite the absence of MRI abnormalities. Furthermore, children with APA-positive ISS may represent an earlier stage of autoimmune hypophysitis in which GH reserve is still normal on provocative testing, but with time may develop into full GHD.

De Bellis A, Salerno M, Conte M, et al. Antipituitary Antibodies Recognizing Growth Hormone (GH)-Producing Cells in Children with Idiopathic GH Deficiency and in Children with Idiopathic Short Stature. J Clin Endocrinol Metab. 2006; J Clin Endocrinol Metab. 2006;91:2484–2489.

Editor's Comment: *The authors pointed out the need for further testing in larger populations, as their study was not designed to establish predictive and/or pathogenic roles of APA. Nonetheless, this paper provides compelling data and a very plausible model that justifies pursuing this line of research. Endocrinologists certainly have precedent in using antibody titers to try to predict hormonal dysfunction and understand disease pathogenesis in disorders of the pancreas,[1,2] adrenals,[3] and thyroid.[4] From a practical perspective, measuring APA titers is a far more appealing diagnostic test than the inaccessible pituitary biopsy, for both clinicians and their patients in search of a diagnosis, with the default option of an "idiopathic" non-diagnosis. Hopefully, APA testing will be available to clinicians soon.*

Adda Grimberg, MD

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Impaired Cognitive Function in Congential Adrenal Hyperplasia

Cognitive function in individuals with congential adrenal hyperplasia (CAH) is a topic of considerable interest. Effects of the condition or its treatment on cognitive function are plausible, ie, a permanent influence of sex steroid hormones *in utero* on brain development, the genetics of CAH or allied alleles, or the effects of under- or over-treatment with glucocorticoids during early postnatal period. Johannsen and colleagues conducted a case-control study of cognitive function in adult women with CAH. Participants included 35 women (84% of the eligible sample) diagnosed with CAH between 1953 and 2003 at a university hospital in Denmark. The patients with CYP21 mutations were grouped into salt wasters (SWs; n = 19, mean age, 31.2 yr; range, 19–46 yr), simple virilizers (SVs; n = 6, 34.6 yr, 23–51), late-onset (LO) CAH (n = 5, 25.5 yr, 19–36) and a mixed group of patients (mixed; n = 5, 28.8 yr, 17–49) with steroidogenic acute regulatory protein (StAR) deficiency (n = 3), CYP21 deficiency diagnosed in adolescence (n = 1), and 17-hydroxylase deficiency (n = 1). Patients with CYP21 deficiency were categorized by mutation severity, salt-wasting status, and clinical presentation. Control group participants were recruited through a general population registry of women born in the same month and year as the patient (response rate = 38%). The woman with the closest match on education was selected for pair-wise matching with the index patient. All participants received a medical interview, physical examination, psychological interview, cognitive assessment, hormone analyses and personality, sexual, and social functioning questionnaires. Five subtests (3 of 6 Verbal and 2 of 5 Performance) from the Wechsler Adult Intelligence Scale (WAIS) provided 3 indices of intelligence (IQ): full-scale IQs (FSIQ), performance (PIQ), and verbal (VIQ). (WAIS IQ scores are defined to yield a population mean of 100 [SD = 15].) Examiners were not blinded with respect to patients' diagnoses.

The combined CAH patient group achieved significantly lower FSIQ (84.5 vs 99.1), VIQ (86.6 vs 97.3) and PIQ scores (85.7 vs 101.3) than the pair-matched control group (Table). The same pattern was true for the SW subgroup. The LO patients also achieved significantly lower FSIQ and VIQ scores than matched controls. Further, the mixed group received significantly lower scores than controls on all IQ indices. In contrast, the SV subgroup was not statistically different from control participants. The SW group received significantly lower FSIQ and VIQ than the SV group, and a nonsignificant trend was observed for PIQ. Patients with verified hyponatremic crises (n = 14) vs all other CAH patients (n = 21) revealed significantly lower FSIQ (78.6 vs 88.4) and VIQ (79.9 vs 91.0), but not PIQ (82.9 vs 87.6).

Full-scale, verbal, and performance IQs in patients with CAH and matched controls.

	n	Full-scale IQ [mean ± SEM (range)]	Verbal IQ [mean ± SEM (range)]	Performance IQ [mean ± SEM (range)]
All CAH patients	35	84.5 ± 2.1 (62–114)[a]	86.6 ± 2.0 (64–107)[a]	85.7 ± 2.4 (62–127)[a]
All controls	35	99.1 ± 2.1 (67–133)	97.3 ± 2.1 (70–132)	101.3 ± 2.0 (73–122)
Salt-wasting CAH	19	81.2 ± 3.2 (62–114)[b]	84.7 ± 2.8 (66–107)[b]	81.5 ± 3.6 (62–127)[a]
Salt-wasting CAH controls	19	96.5 ± 2.6 (67–113)	95.4 ± 2.8 (70–115)	99.1 ± 2.7 (73–120)
Simple-virilizing CAH	6	92.8 ± 2.9 (83–103)	95.5 ± 3.6 (84–103)	91.3 ± 5.1 (73–105)
Simple-virilizing CAH controls	6	95.7 ± 3.6 (85–110)	92.7 ± 2.0 (86–101)	100.0 ± 5.4 (84–120)
LO CAH	5	91.6 ± 4.0 (79–104)[b]	90.0 ± 3.6 (78–99)[b]	96.2 ± 4.0 (87–110)
LO CAH controls	5	105.6 ± 5.6 (92–124)	104.6 ± 4.4 (94–119)	105.0 ± 5.6 (91–122)
Mixed CAH[c]	5	80.0 ± 3.7 (67–88)[b]	79.4 ± 4.7 (64–90)[b]	84.8 ± 2.9 (78–92)[b]
Mixed CAH[c] controls	5	106.2 ± 7.7 (92–133)	102.6 ± 8.5 (85–132)	107.8 ± 4.1 (99–122)

Significance levels for differences between patients and matched controls are indicated as [a] $P < 0.001$ or [b] $P < 0.05$.
[c] Mixed CAH: three patients with StAR deficiency, one patient with 21OH deficiency diagnosed in adolescence, and one patient with 17OH deficiency.

Johannsen TH, Ripa CPL, Reinisch JM, Schwartz M, Mortensen EL, Main KM. Impaired cognitive function in women with congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2006;91:1376–1381.

Editor's Comment: *Pediatric endocrinologists have traditionally been taught that cognitive function and IQ in patients with CAH are not usually sources of concern. This study, and others,[1,2] suggest the contrary: individuals with CAH, particularly the SW-variant, are at risk for lower IQ. Elevated prenatal androgen exposure may affect later patterns of cognitive development and cerebral lateralization, thus individuals with CAH may exhibit a male-typical pattern of cognitive strengths and hemispheric lateralization,[3-5] although other research challenges this conclusion.[6]*

This paper underscores the importance of partitioning the sample in data analyses according to genetic mutation and clinical sequelae. In particular, those individuals who had suffered multiple hyponatremic episodes should be considered a particularly high-risk group for neuropsychological sequelae. Although these investigators grouped CAH participants into categories according to corticosteroid replacement dose, it is puzzling that accompanying analyses were not reported. Nevertheless, the authors noted that glucocorticoids are important for normal maturation of the developing central nervous system and that excessive doses in infancy (reduced in current treatment recommendations[7]) might be partially responsible for the pattern of intellectual deficits observed in this cohort.

This study and corroborating findings underscore the importance of surveilling cognitive function among children born with CAH. To this condition, one could add Turner, Noonan, and Klinefelter syndromes, congenital hypothyroidism, children born small for gestational age, early-onset diabetes, and many others who frequent pediatric endocrinology clinics. Forging collaborations between pediatric endocrinology and hospital-based pediatric psychology or child psychiatry programs that can offer neuropsychological evaluations would likely spare many youths (and their families) needless academic failure and frustration.

David E. Sandberg, PhD

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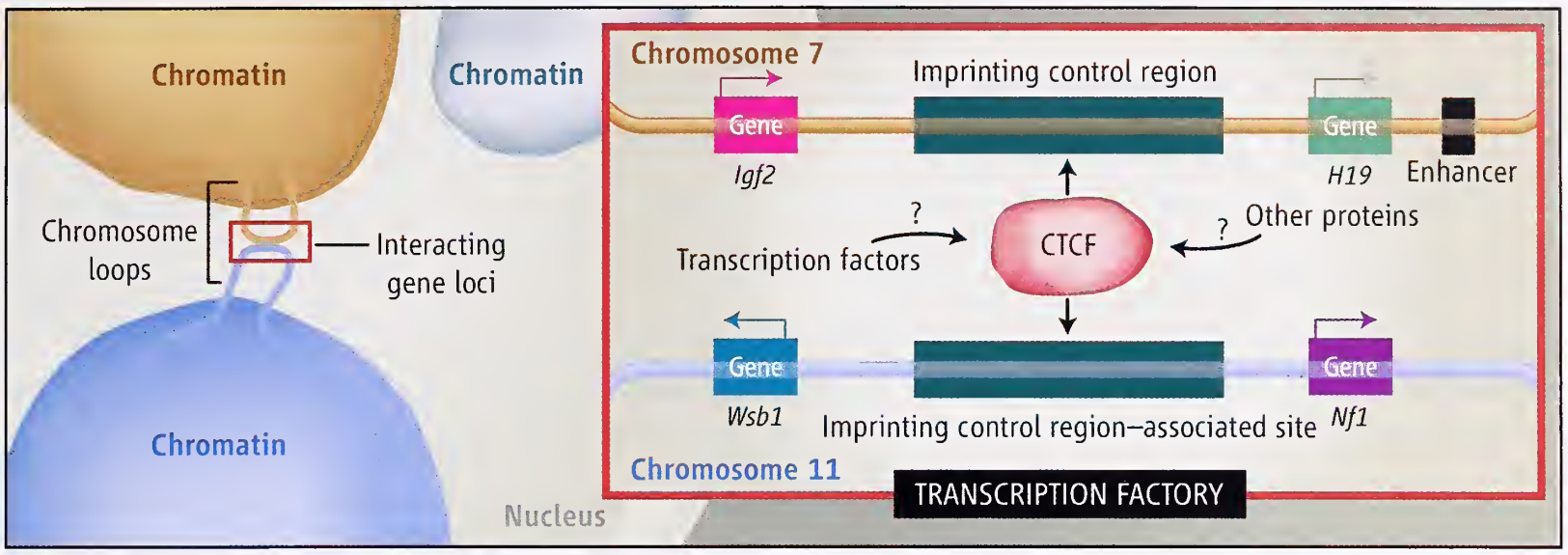
Imprinting, Transcription Factories, and Igf2 Regulation

An important regulator of fetal growth, insulin-like growth factor 2 (Igf2), has received much attention in recent years because it is imprinted, ie, expressed only from the paternal allele, in contrast to the Igf2 receptor, which is expressed only from the maternal allele. New clues regarding regulation of *Igf2* expression have emerged as further insight is gained into how gene expression is regulated in general and how DNA and chromosomes are organized in the nucleus.

As commented upon by Spilinakis and Flavell, DNA in higher organisms is organized with nucleoproteins into different kinds of chromatin from which chromosomes are constructed. Each chromosome resides in a specific region of the nucleus except during cell division.



Interchromosomal rendezvous. The interaction between two different gene loci on two different chromosomes is mediated by the transcriptions regulatory factor CTCF and perhaps other factors. This may occur on regions of the nucleus that are enriched with transcription machinery whereby the genetic elements on one chromosome regulate expression of genes on the partnering chromosome.

Genes being actively expressed typically loop out from condensed chromatin into regions called "transcription factories" where the transcriptional machinery including factors that initiate and regulate transcription resides. Conventionally, this process was thought to be controlled by regulatory elements on the same chromosome as the gene being regulated—so-called *cis* regulation. However, there is growing evidence for genes on one chromosome being regulated by regulatory elements located on a different chromosome, ie, *trans* regulation, and this may help to explain the control of *Igf2* expression. A publication by Ling et al shows that a maternal gene locus on (mouse) chromosome 7 harboring 2 adjacent imprinted genes localizes with a paternal locus on chromosome 11 containing different genes in a manner that depends on a protein termed CTCF (CCCTC-binding factor).

More specifically, *Igf2* and *H19* are coordinately regulated, imprinted genes located ~ 80 kb apart on mouse chromosome 7 (Figure). An imprinting control region (ICR) located between them contains 4 binding regions for CTCF, a zinc finger-binding protein. Using a technique called chromosome conformation capture combined with fluorescence in situ hybridization, Reik and colleagues recently demonstrated that on the paternal chromosome, a differentially methylated region (DMR) loops out to interact with the methylated ICR pushing the *Igf2* promoter into contact with the *H19* enhancer resulting in *Igf2* expression.[1] On the maternal chromosome, a DMR interacts with the unmethylated ICR, partitioning the *Igf2* promoter into a silent loop.

Ling et al applied additional assays to show an interaction of DNA sequences mapped to ICR between the *Igf2* and *H19* loci on chromosome 7 with sequences that mapped to a region located between 2 genes—*Wsb1* and *Nf1*—on chromosome 11, which they called the ICR-associated site. They showed that CTCF binds only to the maternal allele of the *Igf2* ICR and only to the paternal allele of ICR-associated site on chromosome 11 and that these specific interactions are required for co-localization and presumed interaction of relevant intrachromosomal loops from chromosomes 7 and 11.

Ling et al caution that while they cannot be certain, their evidence strongly argues that the genes on chromosomes 7 and 11 physically interact and regulate each other's expression. They note that transcription factories rich in preassembled transcription complexes are presumed to exist within the nucleus and suggest that since there are most likely fewer factories than transcribed genes, some genes would need to share a common factory. Given the strict parental allele specificity of CTCF binding, they further suggest that interchromosomal association plays an important role in the imprinting process. Spilinakis and Flavell propose in their commentary that interchromosomal interactions may be a general phenomenon in gene regulation.

Spilinakis CG, Flavell RA. Managing associations between different chromosomes. Science. 2006;312:207–208.

Ling JQ, Li T, Hu JF, et al. CTCF mediates interchromosomal co-localization between *Igf2/H19* and *Wsb1/Nf1*. Science. 2006; 312:269–272.

Editor's Comment: *These papers add another chapter to the saga of imprinting and further insights into the complexity of gene regulation in higher organisms. It almost makes one long for the days when regulation could be explained one gene at a time with a relatively small number of transcription factors that turned them on and off. The new insights, however, provide a means to begin to explain observations, such as variable expression of genetic disease that we have never understood very well.*

William A. Horton, MD

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IGF-I During High-dose Growth Hormone Treatment of Children Born SGA

Growth hormone (GH) treatment in short children born small for gestational age (SGA) results in a significant improvement of final height, as most children reach normal stature defined as height above –2SD. This treatment is accepted by health authorities in both the US and Europe. However, a previous study by these authors had shown that only a slight and non-significant difference in adult height was observed when 2 GH doses (1 mg/m^2/d [0.033 mg/kg/d] and 2 mg/m^2/d [0.067 mg/kg/d]) were compared.[1] Other reports have shown that the serum insulin-like growth factor (IGF)-I and IGF binding-protein (IGFBP)3 levels are particularly related to the GH dose. Therefore, this study aimed to document GH levels during an overnight profile and IGF-I / IGFBP3 levels before and after 6 months of treatment. This was performed in view of multiple epidemiological studies pointing at cancer risk in relation to high circulating GH and IGF-I levels.

Thirty-six prepubertal short children born SGA were stratified according to gender, and randomized into 2 groups according to dose of GH. The overnight GH profile after subcutaneous injection and IGF data were recorded before and after 6 months of treatment. Results were converted to SDS values when appropriate. Both groups had comparable baseline data. The growth response was significantly higher in the high-dose group after 6 months of treatment. This group had significantly higher GH levels overnight, whether considering area under the curve, mean GH, or maximal GH levels. IGF levels increased in the low-dose group from –1.6 to 0.2 SD, while the change in the high-dose group was from –1.6 to 1.5 SD. In the latter, 74% of the children had IGF-I levels in the highest quartile (>0.84 SD) and 37% had levels above +2SD, compared with only 19% and 6% respectively, in children treated with the lower dose of GH treatment.

The short SGA children given the high dose of GH had evidence of higher circulating GH and IGF-I levels. The IGF-I/IGFBP3 ratio was also more elevated, suggesting higher values of circulating free IGF-I. A higher stimulation of the growth control axis for 6 months produced a significant increase in height, +0.7 SD as compared to +0.5 in the low-dose group. Of interest is that no correlation could be found between the growth response and the increase of GH or IGF-I/IGFBP3 levels. The authors suggested that this may reflect a reduced GH/IGF-I receptor sensitivity, eventually related to the SGA condition, and they recommended monitoring IGF-I levels during GH treatment to ensure that these remain within the normal range for age.

van Dijk M, Mulder P, Houdijk M, et al. High serum levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) during high-dose GH treatment in short children born small for gestational age. J Clin Endocrinol Metab. 2006;91:1390–1396.

First Editor's Comment: *This is the first study comparing 2 doses of GH in short children with SGA and the effect on growth and IGF-I levels. The issue is important since there is a theoretical risk of cancer after prolonged exposure to higher circulating levels of IGF-I and IGF-I/IGFBP3 ratio. This has led to repeated recommendations for the evaluation of circulating IGF-I levels, at least yearly, during GH treatment. The present data document precisely the effect of the 2 most frequently prescribed doses of GH and provide unique data for an appropriate comparison at 6 months of treatment. However, the study does not elucidate the question whether the dose-related increase of IGF values remains the same at a later time, when the high-dose GH does not produce a higher growth rate anymore.*

In any case, the authors challenge the recommended doses and focus on the efficacy of the medication. This paper should be read in relation to the previous study[1] by the same group showing a moderate, but not significant, increase in final height, when doubling the dose up to 0.067 mg/kg/d. It was shown that height gain was dependent on fewer amount of doses over the long-term than over the short-term. Therefore, the economical aspects of long-term administration of higher doses of GH should be considered. Interestingly, the present data again confirmed that during the first 6 months of treatment, there is a significant GH-dose effect allowing better and faster catch-up growth.

Even if a different approach is chosen by individualizing GH treatment to optimize height gain, one may still expect difficulties in adjusting GH dose when taking into account multiple factors such as differences between initial and later treatment periods, dose-related effects on IGF-I, individual susceptibility, and poor correlation between height gain and changes in IGF-I score. It will be of interest to determine whether long-term GH dose adjustments will cope with the observed changes of IGF-I levels and the need for maintaining them within safe limits.

Raphaël Rappaport, MD

Second Editor's Comment: *Although most SGA children show catch-up growth and achieve normal height during the first 2 years of life, approximately 10% to 15% of them remain short with a height below –2 SDS. Recent studies have demonstrated that GH treatment of short children born SGA results in the normalization of height during childhood. Van Pareren demonstrated that long-term treatment of short SGA children with a low dose of GH (1 mg/m^2/d, 0.033 mg/kg/d) was as effective in attaining a normal final height as the treatment with a high dose of GH (2 mg/m^2/d, 0.067 mg/kg/d).[1] In this study van Dijk et al showed that most SGA patients receiving a high dose of GH treatment had high GH levels for most of the day and IGF-I levels and IGF-I/IGFBP3 ratios in the upper quintile. In recent years,*

concern regarding the detrimental effects of persistent high serum GH and IGF-I levels has been expressed in various studies. Of particular importance are the reports of an increased cancer risk (ie, breast, prostate, and colon cancer) in patients with IGF-I levels in the upper tertile to quintile, more so if accompanied by low IGFBP3 levels.[2-4] Recent studies have recommended beginning GH treatment of short SGA children at an early age.[2-4] Thus, GH and IGF-I levels may be elevated in many of these patients for a good part of childhood and adolescence, possibly placing them at an increased risk for complications later in their lives. The long-term deleterious effects of GH treatment in SGA children remain unknown. However, the use of an initially lower GH dose, which can then be individually adjusted and the monitoring of IGF-I and IGFBP3 during GH therapy, in an attempt to maintain IGF-I concentrations in the upper half of the age-adjusted reference range, is strongly recommended.

Roberto Lanes, MD

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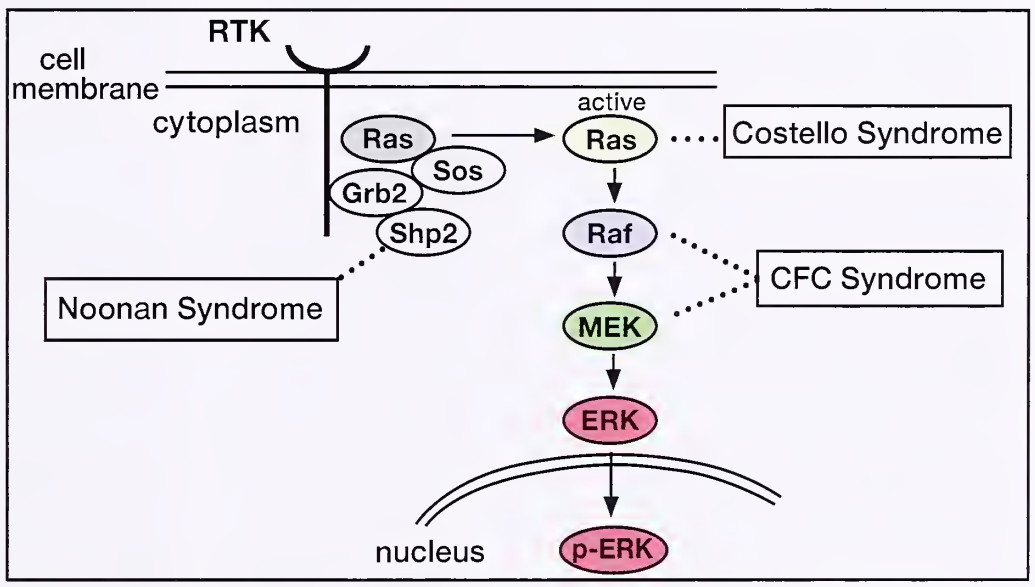
Germline KRAS, BRAF, and MAPK Mutations in Noonan and Cardio-Facio-Cutaneous-Syndrome

The mitogen-activated protein kinase (MAPK) intracellular signal transduction system is one of several signaling systems employed by growth hormone, prolactin, epidermal growth factor, and other mitogens (Figure). The MAPK pathway is important for cell proliferation, growth, aging, and apoptosis. After a growth factor binds to its specific receptor, GRB2 (growth factor receptor-bound protein 2; chromosome 17q23-q25, OMIM 108355), a cytosolic adaptor protein with SH2 and SH3 domains, complexes with the cytoplasmic domain of the activated growth factor receptor. Subsequently, GRB2 interacts with PTPN11 (protein-tyrosine phosphatase, non-receptor type 11; chromosome 12q24.1, OMIM 176876) through SH2-SH3 bonding and then binds to the guanine nucleotide exchange factor-SOS1 (son of sevenless drosophila, homolog 1; chromosome 2p22-p21,OMIM 182530) to mediate growth factor-induced activation of RAS (rat sarcoma viral oncogene homolog; chromosome 11p15.5, OMIM 190020). The RAS family of GTP-binding proteins includes KRAS, NRAS, and HRAS, all composed of 189 non-identical amino acids. After activation by addition of GTP, RAS initiates signal transduction through a series of 3 tyrosine-serine/threonine kinases (phosphorylases) that culminates in phosphorylation and activation of several transcription factors such as activating protein-1 (AP-1), and signal transducer and activator of transcription (STAT) 5. Intrinsic RAS GTPase assisted by GTPase activating proteins degrades RAS-linked GTP to GDP, thus decreasing RAS signaling and depressing the activity of the MAPK pathway. The intermediary kinases in the MAPK pathway include in sequential order:



Ras/Raf/MEK/ERK signal transduction pathway and associated genetic syndromes. Noonan syndrome has also been associated with (K)RAS.
Shp2=PTPN11, MEK=MAP1K1 or MAP1K2, ERK=MAPK3 or MAPK1
Reprinted with permission: Rodriguez-Viciana P, et al. Science. 2006;311:1287-1290.

- BRAF (V-RAF murine sarcoma viral oncogene homolog B1; chromosome 7q34, OMIM 164757) (there are additional RAF isoforms: ARAF and CRAF);
- MAP2K1 (mitogen-activated protein kinase kinase 1; chromosome 15q21, OMIM 176872) and related MAP2K2 (mitogen-activated protein kinase kinase 2; chromosome 7q32, OMIM 601263);
- MAPK3 (mitogen-activated protein kinase 3; chromosome 16p11.2, OMIM 601795) and related MAPK1 (mitogen-activated protein kinase 1; chromosome 22q11.2, OMIM 176948).

MAPK3 in turn phosphorylates AP-1, STAT-5, and other transcription factors. With somatic single point mutations at codons 12,13 or 61, RAS intrinsic GTPase activity is

diminished and the RAS proteins retain GTP, permitting them to become oncogenic by generating unbridled intracellular signaling that leads to unregulated cell proliferation and hematologic, lung, intestinal, pancreatic, thyroid, gonadal, and other neoplasms. Mutations in several of the genes involved in MAPK signaling have been identified and associated with clinical disorders.

Noonan syndrome (OMIM 163950) is an autosomal dominant disorder characterized by a "Turner-like" face, short stature, webbing of the neck, and right-sided anomalies of the heart as well as deafness, motor delay, and a clotting disorder. In approximately 45% of patients with Noonan syndrome, germline heterozygous gain-of-function missense mutations in *PTPN11* have been identified.[1] PTPN11 (also designated SHP2) is an intracellular protein tyrosine phosphatase; adjacent to its catalytic domain are 2 tandem SRC homology 2 (SH2) domains that permit PTPN11 to bind to other proteins with SH2 and SH3 domains and to remove phosphate groups from specific phosphotyrosine residues. Among the substrates of PTPN11 is GRB2. Activating mutations in the SH2 or protein tyrosine phosphatase domains of *PTPN11* increase signal transduction through the MAPK pathway leading to the clinical manifestations of Noonan syndrome, although the cellular mechanism(s) by which they occur is (are) unknown at present.[1] (Heterozygous gain-of-function mutations within the protein tyrosine phosphatase domain of *PTPN11* have also been identified in the LEOPARD syndrome [OMIM 15100], an autosomal dominant disorder with café-au-lait spots and lentigines as well as features similar to those of Noonan syndrome.)

The Costello or facio-cutaneo-skeletal syndrome (OMIM 218040) is characterized by short stature, excessive skin of the neck (webbing), fingers, palms, and soles, curly hair, perioral and perinasal papillomata, developmental delay, and increased susceptibility to neoplasia. In the majority of patients with Costello syndrome, heterozygous gain-of-function mutations in *HRAS* (V-HA-RAS-Harvey Rat Sarcoma Viral Oncogene Homolog; chromosome 11p15.5, OMIM 190020) (*v.i.*) have been found.[2]

The 3 articles presently reviewed document overlapping clinical manifestations and mutations in several genes within the MAPK signal transduction pathway. Schubbert et al report that the clinical manifestations of Noonan syndrome can also arise as a consequence of gain-of-function mutations in *KRAS* (V-KI-RAS2 Kirsten Rat Sarcoma 2 Viral Oncogene Homolog, chromosome 12p12.1, OMIM 190070), a gene "downstream" of *PTP11*. They identified *de novo* germline *KRAS* mutations in 5/174 subjects with Noonan syndrome without *PTPN11* mutations. The most common mutation (present in 3 patients) was substitution of isoleucine for valine at amino acid 14 (Val14Iso); this mutation depressed intrinsic GTPase activity of *KRAS*.

The cardio-facio-cutaneous syndrome (OMIM 115150) is associated with congenital heart disease (pulmonic stenosis, atrial septal defect, hypertrophic cardiomyopathy), distinctive face (high forehead, bitemporal narrowing, hypoplastic supraorbital ridge, depressed nasal bridge, angulated ears), cutaneous abnormalities (sparse hair, ichthyosis-like thickening), and developmental delay. Schubbert et al found a heterozygous mutation in *KRAS* in 1/12 patients with this syndrome. Niihori and colleagues also identified 2 *de novo* germline heterozygous mutations in *KRAS* in 3/43 patients with the cardio-facio-cutaneous syndrome. These investigators further demonstrated 8 heterozygous mutations in *BRAF*-encoding the serine/threonine kinase most immediately responsive to *KRAS* (Figure) in 16/40 patients with the cardio-facio-cutaneous syndrome; 6/8 mutations were localized to the catalytic domain of *BRAF*. The majority of the mutations in *KRAS* and *BRAF* increased signal transduction through the MAPK pathway. These investigators identified no mutations in *PTPN11* in any patient with the cardio-facio-cutaneous syndrome nor did they find aberrations in *KRAS* or *BRAF* in any Noonan subjects. Rodriguez-Viciana and associates found 11 heterozygous gain-of-function *BRAF* mutations in 18/23 patients with the cardio-facio-cutaneous syndrome. They also identified 2 heterozygous, activating mutations in *MAP2K1* and one such mutation in *MAP2K2* in 3/5 patients with this disorder.

Schubbert S, Zenker M, Rowe SL, et al. Germline *KRAS* mutations cause Noonan syndrome. Nature Genet. 2006;38:331–336.

Niihori T, Aoki Y, Narumi Y, et al. Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. Nature Genet. 2006;38:294–296.

Rodriguez-Viciana P, Tetsu O, Tidyman WE, et al. Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. Science. 2006;311:1287–1290.

First Editor's Comment: *The signal transduction pathway and associated genetic syndromes are shown in the figure. Mutations have now been found in several of the protein components of the MAPK signal transduction pathway. That Schubbert et al found 169 patients with clinical manifestations of Noonan syndrome without PTPN11 or KRAS mutations demonstrates the substantial genetic heterogeneity of this disorder and leads one to anticipate the identification of gene mutations in other components of the MAPK signal transduction pathway, perhaps involving SOS, MAPK3, guanosine nucleotide exchange factors, and/or GTPase activating proteins. Indeed, neurofibromin, the neurofibromatosis type 1-associated tumor suppressor product of NF1, is a GTPase activating protein for RAS.*

With the delineation of more and more specific gene mutations leading to clinically described disorders, it may well be time to redesignate such entities according to the gene mutation itself; eg, "Hyperactive RAS disease: type 1, 2 ...," "Hyperactive PTPN11 disease: type 1,2 ...," or according to the genetic pathway involved, eg, "The MAPK syndromes." Indeed, all of the clinical disorders of this pathway share common features to a greater or lesser degree such as short stature, distinctive faces, developmental delay, congenital anomalies of the heart, and skin changes. With intimate knowledge of the basic

abnormalities within the described syndromes, drugs might be devised that ameliorate the hyperactivity of the MAPK pathway and moderate its clinical manifestations. Prenatal diagnosis and perhaps even fetal gene therapy also loom as possible future therapeutic avenues.

Allen W. Root, MD

Second Editor's Comment: *The reader is referred to Vol. 22, No. 2 of GGH for a review of 3 papers dealing with the increased growth hormone resistance of PTPN11 accounting for the short stature of patients with Noonan syndrome.[3] A similar resistance may also be present in other patients with syndromes with or without PTPN11 or KRAS mutations, as they all share common features and short stature. The availability of recombinant IGF-I and IGF-I/IGF BP3 may now allow treatment strategies not previously available.*

Fima Lifshitz, MD

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Idiopathic Short Stature: Psychosocial Development and GH Treatment

Visser-van Balen and colleagues presented a metanalysis of available research on the psychosocial functioning of medically referred children with idiopathic short stature (ISS) and the effects of growth hormone (GH) treatment. Specifically, the authors asked whether or not subgroups of medically referred children with ISS have specific risks and different outcomes when treated. Their search used the Medline and PsycInfo databases and included 11 studies that assessed psychosocial functioning. The results showed that according to parents, short children have lower social competence and more social problems than children with normal stature. The intelligence of the ISS children was within the normal range; however, they functioned on average between normal and below normal. Admittedly, the effect sizes were very small in these studies. Studies on the consequences of being short on psychosocial functioning in adulthood were inconclusive, as none of the adults in the studies had received GH. Two studies reported a relatively low percentage of marriages and relatively high percentage of unemployment and self-reported problems in social functioning among short adults. Other studies have not shown this effect. Of note, most of the studies among children only examined parental records. Studies using teachers and peers did not show lower social competence. Children's own reports regarding self esteem showed relatively few indications of psychosocial problems. The interpretation was that either these children are too young to give an adequate assessment of their own functioning, or they lack time perspective. There were no studies in which similar concepts were studied by both parents and children. The authors speculated that it was possible that medically referred children with ISS had psychosocial problems because they were short. It is also possible that children with psychosocial problems, who were also short, may be referred relatively often. Their conclusion was that medically referred children with ISS had on average more psychosocial problems than children with normal stature.

The review suggested that some risk factors for maladaptation in children with ISS include being teased, being juvenilised, being a boy, having a low intelligence, having a younger but taller sibling, and being part of a low socioeconomic status family. Further studies on the impact of the degree of shortness did not find an effect. This may be because it was not actual height, but perceived height which was crucial in terms of psychosocial risk factors.

Finally, the effects of GH treatment on psychosocial factors were assessed in 9 studies in which the children had a mean height gain of at best 7 cm. On average, GH treatment did not improve psychosocial functioning and only a few studies showed improvement in problem behaviors. Although these pre- to post-treatment assessments with standardized questionnaires did not reveal changes in psychosocial functioning, a retrospective perception of GH treatment by parents and children was generally positive with parents reporting a positive change regarding social functioning and self-esteem of their children.

The 3 main conclusions of this review included: (1) parents of medically referred children with ISS ranked the behavior of their children on average between normal and below normal with more psychosocial problems, (2) some risk factors influencing adaptation in children with ISS have been found, and (3) GH treatment is a means to gain height, but not a means to solve psychosocial problems.

Visser-van Balen, H; Sinnema, G; Geenen, R. Growing up with idiopathic short stature: psychosocial development and hormone treatment; a critical review. Arch Dis Child. 2006;91:433–439.

First Editor's Comment: *This is a very interesting metanalysis, which is probably the first of many subsequent reports to be written concerning ISS and psychosocial functioning. There are many justifiable critiques of the data presented including the lack of control groups, lack of randomization, variable ages at initiation of therapy, and variable duration of treatment. These variables suggest the need for long-term prospective studies in children with ISS for whom treatment is initiated and for whom treatment is not given. It is hoped that one of the GH registries will initiate such a study and that sufficient numbers of children can be*

obtained to be able to adequately assess the influence of these variables on adult psychosocial functioning and adjustment.

William A. Clarke, MD

Second Editor's Comment: *This review of the psychosocial development of medically-referred youths with ISS and the response to GH therapy is notable in that studies are summarized in the context of psychological theory—the disability-stress-coping model.[1] A theory-driven analysis offers the promise of accounting for variability in the experiences of youths with ISS. Most importantly, this strategy generates testable hypotheses regarding the relationship between short stature and quality of life which could be employed in the development of psychosocial treatments serving as an alternative (or adjunct) to medical intervention. Underscoring this point, the authors stated that "hormone treatment is a means to gain height, but not a means to solve psychosocial problems."*

As noted by the authors, the rigor of the research designs employed in assessing the psychosocial adaptation of short youths prior or subsequent to GH treatment is highly variable. Because of this, the studies conducted to date do not support firm conclusions regarding "risk factors" moderating the influence of short stature on psychosocial adaptation. Elements of research design pertinent to psychological studies of short stature have been discussed in this journal.[2]

David E. Sandberg, PhD

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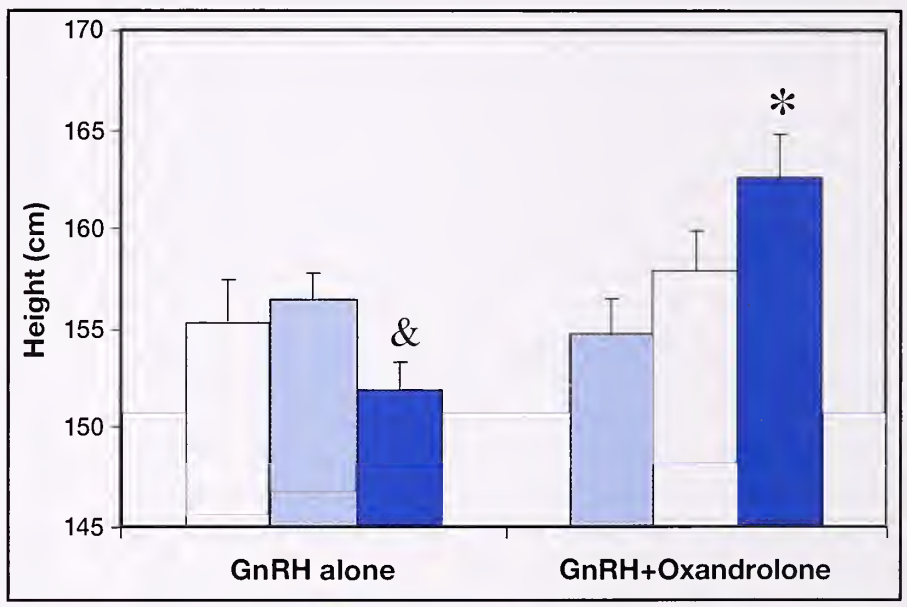
Final Height in Girls with Precocious Puberty Treated with GnRHa and Oxandrolone

Vottero et al assessed the benefits of adding oxandrolone (OX; 0.06 mg/kg/d orally) on the height outcome of girls with central precocious puberty (CPP) who received gonadotropin-releasing hormone analog (GnRHa) treatment (leuprolide acetate, 3.75 mg IM every 28 d) and whose height velocity decreased below the 25th percentile for chronological age. The adult height reached by 10 patients with CPP treated with GnRHa plus OX (group 1) was significantly higher than their pretreatment predicted adult height (PAH) (162.6 ± 2.3 vs 154.8 ± 1.7 cm) and target height (162.6 ± 2.3 vs 158.0 ± 1.9 cm), while 10 subjects with CPP treated with GnRHa alone (group 2) reached an adult height similar to the pretreatment PAH (151.9 ± 1.2 vs 155.4 ± 2.1 cm), but significantly lower than target height (151.9 ± 1.2 vs 156.6 ± 1.4 cm; P<0.005). The difference between final height and pretreatment PAH of patients in group 1 was significantly different from that in group 2 (7.8 ± 2.3 vs −3.8 ± 2.3 cm; P<0.02), as was the difference between final height and target height (4.6 ± 1.8 in group 1 vs −4.2 ± 1.1 cm in group 2; P<0.005) (Figure). No side effects were noted in either group of patients. The authors concluded that combined GnRHa and OX therapy is a viable treatment option for girls with CPP and marked growth deceleration during treatment with GnRHa alone.



□, PAH at start of GnRHa; ▨, target height; ■, final height. Results are shown as mean ± SEM. *, $P < 0.05$ final height of patients treated with GnRHa plus Ox vs. their PAH and target height; &, < 0.05 final height of patients treated with GnRH alone vs. their target height.
Adapted with permission Vottero A, et al. J Clin Endocrinol Metab. 2006;91:1284–1287. Copyright © 2006. The Endocrine Society. All rights reserved.

Vottero A, Pedori S, Verna M, et al. Final height in girls with central idiopathic precocious puberty treated with gonadotropin-releasing hormone analog and oxandrolone. J Clin Endocrinol Metab. 2006;91:1284–1287.

Editor's Comments: *It is well known that in some patients with CPP the growth deceleration during GnRHa therapy may be marked and may preclude an expected improvement in predicted adult height. The addition of growth hormone (GH) to the GnRHa therapy may result in increased final height.[1,2] In this study Vottero et al compared the final height of girls with CPP and growth deceleration while on GnRHa alone, who were subsequently treated with a combination of GnRHa and OX or GnRHa alone. The final height significantly exceeded the target height at the end of the combination treatment and was significantly higher than that of the GnRHa treated girls. Results of this study compare favorably with those obtained in other*

studies[1,2] by the addition of GH to GnRHa. Oxandrolone, a non-aromatizable androgen with a high anabolic to androgenic ratio when compared to testosterone, has been used to stimulate growth in boys with constitutional growth delay and delayed puberty. The OX administration is oral, relatively inexpensive, and devoid of significant side effects. In contrast, GH treatment requires daily subcutaneous injections, is extremely expensive, and its use may be associated with rare, although substantial side effects. This study seems to demonstrate the effectiveness of oral OX for the treatment of patients with CPP whose growth velocities during GnRHa treatment decline significantly; however, studies in a larger number of patients, including boys, will be necessary before this modality of therapy becomes established.

Roberto Lanes, MD

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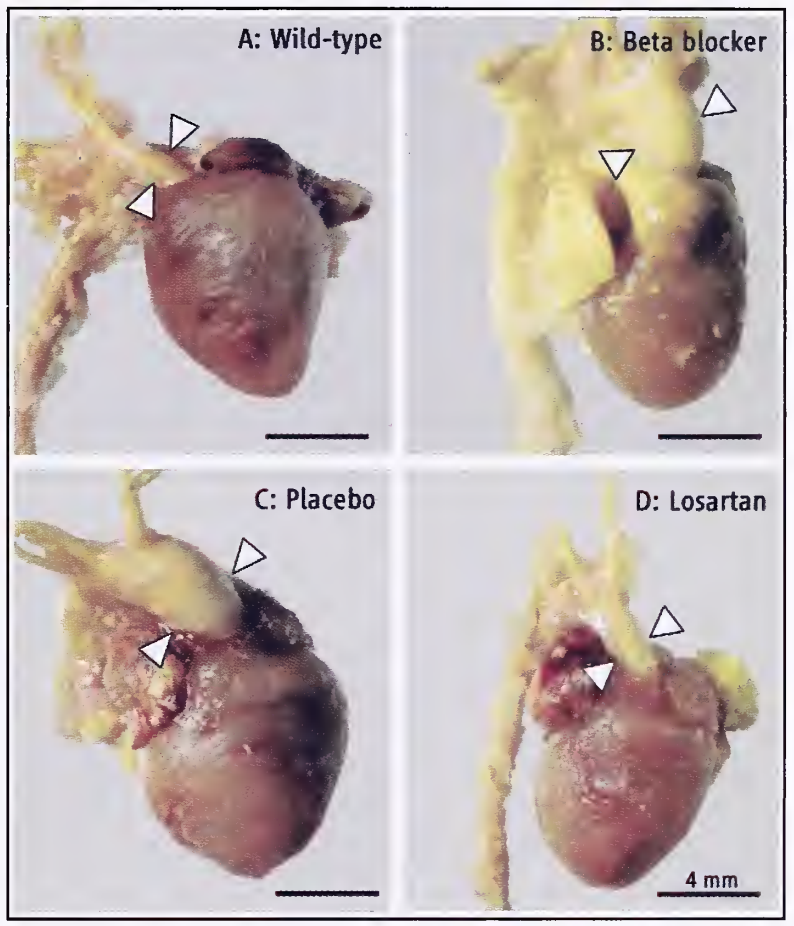
Treatment for Marfan Syndrome

The Marfan syndrome (MFS) was one of the first genetic conditions designated as an inherited disorder of connective tissue. Characterized by abnormalities mainly of the skeleton, eyes and heart, the most serious manifestations involve the aorta, namely, aortic dilatation and aneurysm. Heterozygous mutations of the gene encoding fibrillin-1 (*FBN1*) were identified more than 15 years ago. FBN1 is a principal component of extracellular matrix microfibrils; thus, it was assumed that its function was primarily structural. However, it has recently become apparent that FBN1 binds to and influences the local availability of the growth factor TGF-ß. In fact, evidence has emerged that at least some of the manifestations of MFS reflect excessive TGF-ß signaling. This is because most MFS mutations are believed to reduce FBN1 in tissues; consequently, there would be less FBN1 to sequester TGF-ß and keep TGF-ß signaling in check. Indeed, mice genetically engineered to have reduced tissue levels of Fbn1 exhibit impaired pulmonary alveolar septation associated with increased TGF-ß signaling. This developmental defect can be corrected by administration of antibodies that neutralize TGF-ß signaling. Much of this work has been carried out by a group headed by Dietz at Johns Hopkins. The group has now directed their attention to the role of TGF-ß signaling in causing aortic aneurysm in MFS.

The authors studied mice heterozygous for an *Fbn1* mutation involving a cysteine substitution in one of the Fbn1 epidermal growth factor-like domains; the mutation belongs to the most common class of mutations responsible for MFS. The mutant mice develop progressive aortic root dilatation evident as early as 2 weeks of age; the aortic roots of mutant and normal (wild-type [WT]) mice can be clearly distinguished by ultrasound at 7 weeks. Histologically, the aortic root of the mutant mice exhibits aberrant thickening of the media with disarray of elastic fibers and increased collagen deposition. Cells within the aortic media of the mutant mice also exhibit nuclear staining for phosphorylated Smad2 (pSmad2), which is only minimally detected in the WT mouse aortic root. Since phosphorylation of Smad2 and nuclear translocation pSmad2 are critical steps in TGF-ß signal transduction, detection of nuclear pSmad2 indicates TGF-ß signaling activity in these cells.



Heart of the matter. The aorta (arrows) of a normal mouse (A) and a losartan-treated mouse with a fibrillin-1 mutation (D) are indistinguishable, but those of mutant mice treated with a beta blocker (B) or placebo (C) have aneurysms.
Reprinted with permission: Travis J. Science. 2006;312:36–37.

The 7-week-old mutant mice were treated with placebo or low- or high-dose TGF-ß neutralizing antibody. After 8 weeks of treatment, aortic root growth was no different between both antibody-treatment groups and WT controls in contrast to continued dilatation in placebo-treated mice. Histology revealed substantial normalization of vessel architecture with loss of pSmad nuclear staining in both antibody-treatment groups. These data were considered consistent with the notion that TGF-ß signaling contributes to aortic root dilatation in this mouse model and that TGF-ß antagonism represents a potential treatment strategy for aortic disease in MFS.

The group became interested in the drug losartan, an angiotensin II type I receptor (AT1) antagonist, not only because it lowers blood pressure—a desirable effect

in patients with aortic aneurysm—but also because it antagonizes TGF-ß in some circumstances. Accordingly, they initiated a therapeutic trial to determine if losartan could prevent the formation of aortic dilatation in the mutant mice. Either losartan or placebo was administered at 2-weeks gestation and continued until 10 months of age. To distinguish the effects of lowering blood pressure from those due to TGF-ß antagonism, the ß-adrenergic blocker propranolol was given in doses that caused hemodynamic effects comparable to those of losartan. An important advantage of using propranolol as a control is that it is commonly employed to slow aortic growth in MFS. Upon analysis, aortic root dilatation with wall thickening and elastic fiber fragmentation was detected in the placebo- and propranolol-treated mutant mice, but not in the losartan-treated mice whose aortic root measurements were virtually indistinguishable from those of WT littermates (Figure).

A postnatal trial was also done since MFS is typically diagnosed after birth and also because losartan is contraindicated during pregnancy. The researchers compared placebo, propranolol, and losartan in postnatal mutant mice beginning at 7 weeks of age at which time the aortic root diameter was greater than for WT untreated mice. After 6 months of treatment, they observed that losartan treatment prevented elastic fiber fragmentation, which was found for placebo- or propranolol-treated mice. Aortic root growth was partially normalized by propranolol, but it was indistinguishable from WT controls for mice treated with losartan. Losartan-treated mice, but not propranolol-treated mice, showed a blunting of TGF-ß signaling in the aortic media cells. In short, the aortic root of losartan-treated postnatal mutant mice was comparable to that of WT control mice.

The group then showed that the distal alveolar airspaces in the lungs of postnatal losartan-treated mutant mice had sizes close to WT controls in contrast to placebo-treated mutant mice whose airspace measurements were increased, as was expected for the mutant mice. This finding provided further evidence that the losartan effect on the aortic root is mediated by its antagonism of TGF-ß rather than some unappreciated hemodynamic effect; although the authors conceded that the mechanism by which AT1 blockade antagonizes signaling is not known.

Finally, the authors discussed the potential use of losartan for treatment of MFS. They point out that losartan is currently in widespread use for treatment of hypertension and prevention of strokes in both adults and children. In an accompanying editorial, Travis states that the NIH is finalizing plans for a multicenter clinical trial of losartan for children and young adults with MFS.[1]

Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312:117–121.

Editor's Comment: *The results reported in this paper are both exciting and promising. As noted, prospects for effectively treating MFS were not good when it was viewed as a disorder of extracellular matrix structure. However, in its new light as a disease mediated at least in part by excessive signaling by a growth factor, the possibilities are much better as illustrated here. The authors showed in the mouse that TGF-ß antagonism can ameliorate manifestations of MFS in 2 organ systems: cardiovascular and lung. One wonders about disturbances of other organ systems, such as skeletal overgrowth. This work is a great example of successful translational research.*

William A. Horton, MD

Reference

1. Travis J. Science. 2006;312:36–37.

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In This Issue
Reviews & Comments

E-Reviews & Comments

MOLECULAR PATHOGENESIS OF ACHONDROPLASIA

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INTRODUCTION

Achondroplasia (OMIM 100800) is by far the most common chondrodysplasia in humans with an estimated prevalence to be one in 15 000 to 40 000 live births. It is the prototype of short-limbed dwarfism and the archetype of a group of disorders that range from the much more severe thanatophoric dysplasia (TD) to the less severe hypochondroplasia.[1] These disorders share a common qualitative clinical phenotype dominated by short limbs, long trunk, large head with frontal bossing, and midfacial hypoplasia.[2]

Infants with achondroplasia typically present with mild-to-moderate limb shortening, moderate craniofacial manifestations, and a lumbar gibbis. These features typically become more noticable over time. The gibbis usually gives way to a lumbar lordosis, and infants and children with achondroplasia are at risk for spinal cord compression at the foramen magnum, as well as obesity. Average adult height for men with achondroplasia is 131 ± 5.6 cm; for women it is 124 ± 5.9 cm.

Thanatophoric dysplasia is much more severe in general. It is usually lethal in the perinatal period, but on rare occasions infants survive with a poor prognosis. Craniofacial abnormalities are much more dramatic. The thorax appears long but narrow and is associated with severe respiratory distress. Two types of TD (TDI and TDII: OMIM 18700 and 18760) can be distinguished radiographically. SADDAN dysplasia refers to a clinical phenotype

From The Editor's Desk

Dear Colleague:

The latest issue of GGH includes the highlights of 2 important annual meetings in our field. The printed journal contains the highlights of the Endocrine Society's meeting held in June in Boston. The online journal also contains highlights from the European Society of Pediatric Endocrinology meeting held in July in Rotterdam. The lead article by Dr. William A. Horton, "Molecular Pathogenesis of Achondroplasia," elucidates the advances that have occurred in the understanding of the mechanisms of growth alterations of these patients. A look at the future with potential therapeutic considerations adds value to the clarification of the pathophysiology of the disease. Additionally, there are 17 reviews of recent papers that were selected by the Editorial Board. Altogether, the journal will stimulate you and enhance your continuous medical education efforts. I am very pleased to note that we continue to expand the content and size of the e-reviews; for example, this issue contains 11 reviews of papers with editorial comments. As well, new clinical practice guidelines continue to be added to the website. In order to provide more reviews, the index of volume 22 (2006) is now only online. Moreover, all issues and subjects are searchable online.

Finally, it is the time of year that I take the opportunity to wish you all the best for the holiday season and best wishes for the New Year.

Sincerely,
Fima Lifshitz, MD

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intermediate in severity between TD and achondroplasia accompanied by developmental delay and acanthosis nigricans.[3] Patients with hypochondroplasia (OMIM 146000) typically present in mid childhood with mild short stature and a stocky build; the craniofacial manifestations may be minimal. Patients with hypochondroplasia blend in to the lower range of normal stature; many go undiagnosed or may be considered idiopathic short stature or be confused with another bone dysplasia.

GENETICS

Achondroplasia was mapped to chromosome 4p16.3 in 1994, and heterozygous mutations of Fibroblast Growth Factor Receptor 3 (*FGFR3*) were identified shortly afterwards.[4-6] *FGFR3* mutations were subsequently discovered for the TDs and hypochondroplasia (Figure 1).[7-9] Remarkable degrees of genetic homogeneity and genotype:phenotype correlation soon became apparent as virtually all patients with classic achondroplasia were found to have the same Gly380Arg mutation in the transmembrane domain of this tyrosine kinase receptor.[1,8] Similarly, all infants with TDII had the identical Lys650Glu mutation in the distal kinase domain, whereas an Asn540Lys mutation in the proximal kinase domain was detected in most patients with hypochondroplasia.[7-9] Almost all infants with TDI have mutations that introduce free cysteine residues in the proximal extracellular ligand-binding domain of the receptor. Of note is that mutation of lysine 650 can produce 3 different clinical phenotypes: conversion to glutamic acid results in TDII, conversion to methionine causes SADDAN, and conversion to serine leads to hypochondroplasia.[10,11]

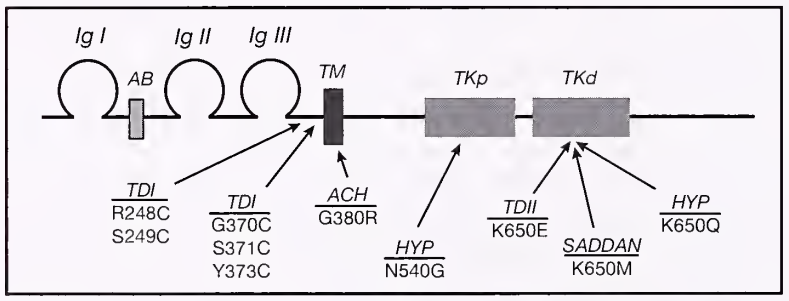

Figure 1. Domain structure of FGFR3 and major sites of mutations. *Ig*: immunoglobulin, *AB*: acid box, *TM*: transmembrane, *TKp/d*: proximal and distal tyrosine kinase domains, *ACH*: achondroplasia, *HYP*: hypochondroplasia, *TD*: thanatophoric dysplasia, *SADDAN*: severe achondroplasia with developmental delay and acanthosis nigricans.

The penetrance of the achondroplasia mutation is 100%, meaning that individuals with *FGFR3* Gly380Arg mutation have achondroplasia. The vast majority of infants with FGFR3 mutations are born to parents without FGFR3 mutations, although there is a strong correlation with advanced paternal age (over 35 years). These findings were initially attributed to increased mutability of FGFR3 during spermatogenesis. However, recent observations, including the detection of *FGFR3* in all germ cells except for elongated spermatids in adult men and failure to detect sufficiently high mutation rates in sperm from older males, have led to the alternative explanation that sperm bearing mutant *FGFR3* have a selective advantage over sperm bearing normal *FGFR3* receptors.[12-14]

MOLECULAR PATHOGENESIS

a) Receptors

The *FGFR3* encodes one of 4 closely related FGF receptors (FGFR1–4) in mammals.[15] All have an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain that contains a split tyrosine kinase subdomain. The receptors differ in their temporal and spatial distribution of expression. Additional diversity is generated by alternative splicing that influences ligand specificity. Mutations similar to those in *FGFR3* have been observed in *FGFR1* and *FGFR2* in human craniosynostosis syndromes.[16]

After initial speculation that achondroplasia mutations cause loss-of-receptor function, it soon became evident they actually result in gain of *FGFR3* function, and the extent of this gain was found to correlate with the severity of the clinical phenotype.[17] The most compelling evidence came from genetic engineering experiments in mice in whom *FGFR3* was either inactivated or the receptor activated in cartilage by introducing achondroplasia or TD mutations, or by overexpressing ligands that activate *FGFR3*.[18-23] Mice in whom *FGFR3* was inactivated had long bones, while mice with excess *FGFR3* activation had short bones. Accordingly, *FGFR3* mutations associated with achondroplasia are often referred to as activating mutations.

Of interest is the fact that functions which are gained by activating mutations differ, depending on the cell type in which the *FGFR3* receptor is expressed. For instance, *FGFR3* activation promotes mitosis and blocks differentiation in many non-chondrocytic cell types. In fact, activating TD mutations have been found in colon and bladder carcinoma and multiple myeloma, as well as in benign adenoid seborrheic keratoses.[24-27] In growth plate chondrocytes, however, activation of *FGFR3* has the opposite effect as discussed below.

b) Dimerization

The binding of FGF ligands to *FGFR3* monomers leads to receptor dimerization. Which of the 22 known FGFs is (are) the physiologic ligand(s) for *FGFR3* is (are) not known, although FGFs 2, 4, 9 and 18 are probably the best candidates based on the distribution of expression and ability to bind and activate FGFR3 in *in vitro* assays.[28,29] It is also conceivable that different FGF ligands activate *FGFR3* in different physiologic situations. Heparin sulfate-bearing proteoglycans on the cell surface, such as syndecans, as well as alternative splicing of ligand-binding subdomains, influence binding specificity.[30-32]

Dimerization activates the intrinsic tyrosine kinase activity of the receptor and promotes transphosphorylation of

key tyrosine residues in the cytoplasmic domain. These residues serve as docking sites for adapter proteins and signal effectors that are recruited to the activated receptors and which propagate FGFR3 signals.[33-36]

c) Signaling pathways

FGFR3 signals influence a variety of cellular events and processes largely through inducing or repressing expression of target genes in a cell-specific context. Four main signaling pathways have been identified to date to propagate *FGFR3* signals: STAT, MAPK, PLC-γ, and PI3K-AKT (signal transducer and activator of transcription 1, mitogen-activated protein kinase, phospholipase C gamma, phosphatidylinositol phosphate-3-kinase-serine/threonine kinase [protein kinase B]) with the first 2 receiving the most attention.[31,37-42] The most relevant signaling pathways are illustrated in Figure 2. STAT1 signals are thought to induce expression of mitotic inhibitors, such as the cdk inhibitor p21.[40] Using microarrays to assess changes in gene expression in chondrocytic cells, Dailey et al[43] showed that FGFs initiate signals in multiple pathways that result in the induction of antiproliferative functions and down regulation of growth-promoting molecules.

Two MAPK pathways have been implicated, the strongest evidence coming from transgenic mice in whom expression of constitutively active members of the 2 pathways was targeted to cartilage, including growth plate cartilage. Expression of activated MKK6, which specifically activates the MAPK-p38 pathway, inhibits chondrocyte proliferation in part through induction of the transcription factor Sox 9.[44] Chondrocyte hypertrophy was also inhibited in these dwarf mice. Expression of constitutively active MEK1, which specifically activates the MAPK-ERK pathway, produced a similar dwarf phenotype, but through inhibition of terminal chondrocyte differentiation with no inhibitory effect on cell proliferation.[45] These observations underscore the importance of both chondrocyte proliferation and terminal (hypertrophic) differentiation in linear bone growth and the central role of *FGFR3* in negatively regulating these events.

It is important to emphasize that FGFR3 is one of many physiologic regulators that modulate linear bone growth. Its normal function is as a negative regulator. The mutations associated with achondroplasia and related conditions are thought to act through exaggeration or enhancement of this normal physiologic function rather than through acquisition of new functions.

d) Consequences of mutations

Several mutation-specific mechanisms have been proposed to explain how activating mutations of *FGFR3* enhance FGFR3 signals (Figure 3).[1,46] The transmembrane achondroplasia mutation is thought to stabilize FGFR3 dimers following ligand-induced dimerization, although this mechanism has recently been challenged.[47,48] Monsonego-Ornan et al[49] have suggested that this mutation slows receptor internalization, leaving it on the surface to signal. The free cysteine residues introduced by the TDI mutations are believed to form disulfide bonds resulting in dimerization, which in turn activates the receptor.[33]

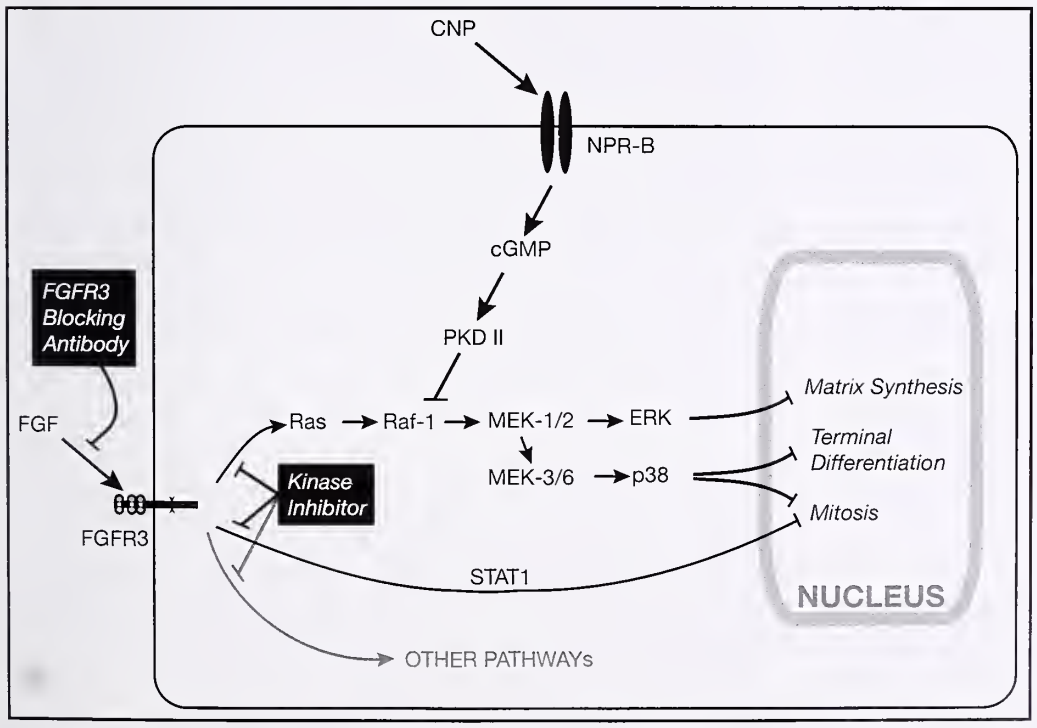

Figure 2. Signaling pathways and potential therapeutic strategies. FGFR3 signals are propagated through STAT1, MAPK-ERK, MAPK-p38 and probably other pathways which inhibit growth plate chondrocyte proliferation, post-mitotic matrix synthesis and terminal (hypertrophic) differentiation. The CNP-NPR-B pathway inhibits the MAPK pathways. Proposed therapeutic strategies include chemical inhibition of FGFR3 tyrosine kinase, antibody blockade of ligand-induced receptor activation, and enhancement of CNP-NPR-B signals.

The mutations of lysine 650 alter the conformation of the kinase domain, constitutively activating the intrinsic enzyme activity to different extents, corresponding with the severity of the clinical phenotype.[34,46,47] It is not clear if receptors carrying the lysine 650 mutations reach the cell surface to become activated by ligand. The receptor tyrosine kinase is also activated by the common (Asn540Lys) hypochondroplasia mutation, but presumably to a relatively low degree, ie, comparable to the Lys650Ser mutation that is associated with a hypochondroplasia phenotype.

A mechanism that seems to be relevant to all of the mutation types is delayed turnover of activated receptor, which increases the overall FGFR3 signal output.[50] Like most other transmembrane receptors, FGFR3 is internalized within endosomes relatively soon after

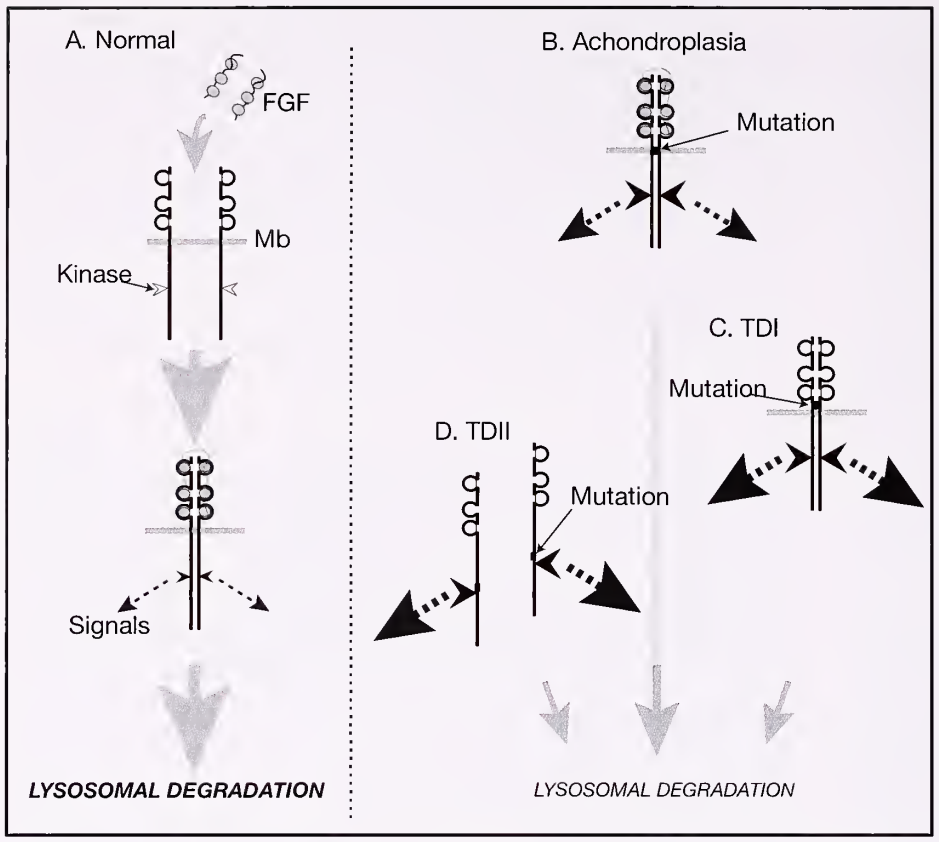

Figure 3. Proposed mechanisms by which mutations lead to gain of FGFR3 function. (A) Normally, ligand induces dimerization of receptor monomers, which activates kinase and initiates propagation of FGFR3 signals. Activated FGFR3 is targeted to and degraded by lysosomes relatively soon after activation. (B) FGFR3 dimers are stabilized by mutation (arrow) in transmembrane domain of the receptor in achondroplasia. (C) FGFR3 dimers are induced by formation of disulfide bonds in the proximal extracellular domain (arrow) in TDI. (D) Kinase is constitutively activated by mutation in TDII (and to lesser extent, in SADDAN and hypochondroplasia). (E) Lysosomal degradation is slowed in all 3 conditions. Mb: membrane.

activation. Since the intracellular "signaling" domain of the receptor has access to cytoplasmic signaling molecules, the endosomal-bound receptor continues to propagate signals until it is eventually degraded in lysosomes. Lysosomal targeting of receptors is mediated by the addition of multiple ubiquitin molecules to the activated receptor; the ubiquitin serves as a "lysosomal targeting signal." FGFR3 ubiquitination is directed by the adapter protein c-Cbl, which functions as a ubiquitin ligase. c-Cbl activation occurs following FGFR3 activation; accordingly, the activated receptor directs its own degradation presumably as a negative feedback mechanism to keep its signaling output in check. In achondroplasia and related disorders, however, there is a defect in c-Cbl-mediated FGFR3 ubiquitination that leads to slowed receptor degradation and consequently, increased signal output.[50]

Another pathway that down modulates FGFR3 signaling involves C-type natriuretic peptide (CNP).[51] Through interaction with its receptor, natriuretic peptide receptor B (NPR-B), CNP induces accumulation of intracellular cGMP (Figure 2). Of interest is that mutations of NPR-B are responsible for acromesomelic dysplasia, type Maroteaux (OMIM 602875).[52] Both CNP and NPR-B are expressed in the proliferative and prehypertrophic zones of the growth plate, setting up a potential autocrine or paracrine regulatory circuit.[52] Considerable evidence suggests that downstream signals from NPR-B antagonize FGFR3 downstream signals. More specifically, an increase in cGMP is known to activate a number of signaling mediators, including cGMP-dependent protein kinases (cGKs, or alternatively PKGs), one of which—cGKII (PKGII)—is thought to inhibit MAPK-ERK signaling at the level of Raf-1.[53,54] Probably most telling is a genetic study in which mice exhibiting dwarfism due to expression of the achondroplasia mutant *FGFR3* transgene in cartilage were mated to mice in whom CNP expression was also targeted to cartilage.[55] The dwarfism of the "achondroplasia" mice was rescued by expression of CNP in cartilage.

THERAPEUTIC CONSIDERATIONS

As the molecular pathways involved in the pathogenesis of achondroplasia and related disorders have become clearer, a number of potential therapeutic strategies have emerged. Most of these approaches are similar to those used to treat cancer. This may seem odd, since the physiologic disturbances are in opposite directions, ie, too much growth in cancer, too little in achondroplasia. However, at the molecular level, the mechanisms are quite similar, ie, too much tyrosine kinase activity.

The most attention in achondroplasia has gone to inhibiting the FGFR3 tyrosine kinase through small chemical inhibitors. This approach has a strong rationale because essentially all of the cellular and higher level physiologic disturbances that interfere with bone growth seem to be driven by the excess in tyrosine kinase activity. For example, even the defect in lysosomal targeting and degradation of the activated receptor appears to be a downstream consequence of increased kinase activity. Selective FGFR3 kinase inhibitors have been developed and show promise in cell and organ culture experiments, but to date none has shown success in whole animals.[56]

An alternative approach has involved generating antibodies to block FGFR3 activation. Although highly specific humanized antibodies have been developed, there have been no reports to date of success beyond cell culture experiments in which they block receptor activation well.[56]

The therapeutic use of CNP or a CNP analog that could activate NPR-B signaling pathway to counter excessive FGFR3 signals transmitted through the MAPK–ERK and possibly MAPK-p38 pathways has been proposed.[55,57] This approach is appealing because other natriuretic peptides have been used clinically for their hemodynamic

effects in adults and even in children.[57,58] While they appear to be safe at least in the short term, a major drawback is their very short half-life requiring them to be administered by infusion, which would not be satisfactory for long-term treatment of achondroplasia.

A variation of this approach involves therapeutically targeting the NPR-C, another natriuretic peptide receptor that binds to CNP. The NPR-C, which is present on hypertrophic chondrocytes in the growth plate,[52] lacks the ability to increase intracellular cGMP and has been proposed to function as a clearance receptor to down regulate the effects of natriuretic peptides.[57] Theoretically, blocking NPR-C would lead to an increase in available CNP to bind to NPR-B in the growth plate, which in turn would be expected to antagonize FGFR3-MAPK-ERK/p38 signals as discussed above.

There are 2 considerations regarding molecular treatment of achondroplasia that deserve special attention. The first is that treatment would need to be long term, probably starting soon after birth when the diagnosis is made and lasting through puberty. This adds challenges to any form of treatment.

The second consideration relates to the difficulty in targeting therapeutic agents to the cartilaginous growth plate. Compared to most tissues, cartilage is avascular and the dense and highly charged extracellular matrix that surrounds chondrocytes represents a formidable barrier for drug delivery. Indeed, these factors may explain at least in part why treatments that have worked in cell and organ culture experiments, have failed in whole animals. Agents given systemically may need to be administered in higher doses than those used for most other tissues in order to achieve therapeutic levels in the growth plate, and this could create a predisposition to side effects in the other tissues. Accordingly, it may be necessary to develop means to target agents to growth plate chondrocytes to reach effective doses of drugs and to avoid adverse effects in other tissues. Concern over such adverse effects may be especially relevant to the central nervous system where FGFR3 is known to be expressed postnatally.[59]

GENETIC IMPLICATIONS

The diagnosis of achondroplasia can usually be made clinically. In rare instances in which the patient is too young or exhibits atypical findings, it can be established by molecular genetic testing for the achondroplasia mutation.[60] There are a number of laboratories that carry out such testing, and these can be accessed through the GeneTest Laboratory Directory at www.genetests.org. Given the virtual 100% penetrance of achondroplasia, the risk to family members who do not display clinical features of achondroplasia, ie, siblings and offspring of affected individuals, as well as siblings of parents, is extremely low and testing is not ordinarily indicated. However, prenatal genetic testing may be useful in situations in which both parents have achondroplasia to identify fetuses with homozygous, or double-dose, achondroplasia. Such matings are at 25% risk for this much more severe form of achondroplasia.

Molecular genetic testing for hypochondroplasia may confirm a suspected diagnosis. However, only about 70% of individuals with typical findings of this condition are heterozygous for a mutation of FGFR3, presumably because mutations in genes other than *FGFR3* can result in the hypochondroplasia clinical phenotype.[61]

The position statement of the Little People of America regarding genetic discoveries in dwarfism may be reviewed online.[62]

CONCLUSION

The tyrosine kinase-mediated transmembrane receptor FGFR3 is an important negative regulator of linear bone growth acting mainly through the STAT1, MAPK-p38, and MAPK-ERK signaling pathways to inhibit chondrocyte proliferation and terminal differentiation in the growth plate. Mutations that enhance these actions produce the qualitative achondroplasia clinical phenotype; the extent of this enhancement correlates with the severity of this phenotype. The mutations act through promoting or stabilizing the dimerization required for receptor activation, by directly activating kinase activity through conformational change of the receptor and by slowing of receptor degradation. Several strategies have been proposed to therapeutically counter the increased FGFR3 signal output, including chemical tyrosine kinase inhibitors and blocking antibodies, both selective for FGFR3 and activation of the CNP-NPR-B-cGMP pathway, which antagonizes MAPK-ERK/p38 signals downstream of FGFR3. All 3 strategies have shown success in cell and organ culture systems, but not yet in whole animal trials, perhaps because they may need to be targeted directly to growth plate chondrocytes to achieve therapeutic effect restricted to growing bones.

Research on achondroplasia and mutations of *FGFR3* has stimulated much interest in the molecular and cellular biology of both normal and abnormal linear bone growth. Indeed, many new genes whose products influence bone growth have been discovered or better delineated in the past several years, as have pathways that contribute to the regulation of bone growth. The hope is that these discoveries will lead to novel, safe, and effective therapies for disorders of linear bone growth within the next several years.

Acknowledgement

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REVIEWS & COMMENTS FROM THE LITERATURE

Highlights of the Endocrine Society's 88th Annual Meeting, Boston MA, June 24-28, 2006

The Endocrine Society's Annual Meeting provided exciting presentations in plenary sessions and symposia, endocrine updates, endocrine debates, and special sessions, including multiple reviews of pediatric interest. However, I could not do justice to encapsulate those presentations in a succinct manner. Thus, only the highlights of the poster and oral presentations of the scientific papers that attracted my attention are summarized, particularly the research focusing on growth.

Adrenals

A novel mutation of melanocortin 2 receptor accessory protein (MRAP) gene was identified in 2 brothers with familial glucocorticoid deficiency by Li Chan et al. The first codon exon TAC-TAA lead to a severely truncated protein at position 11 of the MRAP gene, which rendered the adrenal gland unresponsive to ACTH.

Bone

How much of a work-up is needed when healthy children present with frequent fractures? That was the question addressed by Robert Olney et al. Sixty-nine patients with low-energy fractures were evaluated and compared to 56 controls. Those with fractures did not present low whole body bone mineral density (BMD) and did not ingest lower calcium or vitamin D. The researchers concluded that occult metabolic bone disease was not a common cause of repeated fractures in children.

Low-dose pamidronate treatment of osteoporosis in non-ambulatory children was shown to be effective by Horacio Plotkin et al. They administered pamidronate 4.12 mg/kg/yr intravenously (administered over 2 days every 4 months). There was a significant increase in BMD with treatment without significant side effects.

Recombinant human growth hormone (rhGH) induced a marked and sustained elevation in circulating levels of osteocalcin and c-terminal telopeptide of type I collagen. The increased levels exceeded the impact of bone fracture alone. This was reported by Jens Christiansen et al. The increased levels persisted for up to 12 weeks after cessation of rhGH treatment, suggesting that osteocalcin and type I collagen could be markers of illicit administration of rhGH.

Diabetes

Data on 5928 children with diabetes from 7 pediatric endocrinology centers in the United States were reviewed by Vanessa Davis et al. Approximately 10% had type 2 diabetes mellitus (T2DM). Most of those with T2DM were initially treated with oral medications, but after 5 years with the disease, insulin became the most commonly used therapy. During the 5 year study, only 25% had an improved HbA1C, 46% worsened, 34% developed co-morbidities and complications, and 29% were unchanged. These data pointed out a worrisome trend of poor treatment adherence and an aggressive natural course of T2DM in children.

Growth

Humans with mutations in PROP1 present with hypopituitarism. Luciano Carvalho et al determined the molecular basis of the disease; they compared mice with a mutation of the PROP1 gene with Pit1 mutant mice. The mice with Prop1 mutation showed delayed vascular development, reduced cell proliferation, and elevated rate of apoptosis. These alterations may explain why the pituitary is very small in x-rays and MRI scans in patients with PROP1 hypopituitarism.

Did the small-bodied Hominis from Flores in Indonesia suffer from a molecular defect in the GH receptor (GHR) gene (Laron syndrome)? That provocative question was posed by Zvi Laron. Proposing a diagnosis due to a molecular defect in the GHR for the pathological findings of skeletons unearthed in Indonesia and whose age is 18 000 years is a challenge that may be resolved by DNA analysis; the possibility is interesting.

The response to rhGH therapy in 20 GH-deficient (GHD) patients receiving stimulant medication was assessed by Parm Gill et al. After one year of therapy, the growth rate of children treated with stimulant medication and rhGH was significantly lower than that of control patients with GHD, though the BMI of the 2 groups was similar.

The protein polymorphism of the GHR characterized by deletion of exon 3 has been linked to the magnitude of the response to rhGH treatment. Laura Audi et al demonstrated that the frequency of GHR polymorphism was higher among small for gestational age (SGA) patients than that of a control population with normal height. However, in 2 separate studies, Antonio Carrascosa et al and Veronica Mericq et al showed that in short SGA patients the response to rhGH treatment was not different among those with or without this genomic deletion. On the other hand, Gerhard Binder et al showed that Turner syndrome patients with a deletion of exon 3 presented a significantly reduced increment in height velocity during the first year of therapy with rhGH, as compared with Turner syndrome patients who

did not have this genomic mutation. The differences in height gain persisted until adult height was attained.

As MRI techniques have become more sophisticated, there are incidental findings detected in children undergoing assessment for short stature. Elena Sutu described 44 incidental findings in 38 children. Patients received further evaluations and none required treatment for the incidentalomas.

The prevalence of recurrences of craniopharyngioma in rhGH treated children was reported by Edward Laws et al. There were 51 recurrences among 773 patients with a prior history of craniopharyngioma. The risk ratio over a 7-year period was approximately 7%.

The diagnosis of GH deficiency (GHD) is often based on the response to pharmacological agents that stimulate GH release. Susan Rose and Melissa May demonstrated that children who ingested a dietary electrolyte drink (Diet Sprite™ >15 mL/kg) had improved tolerance to clonidine stimulation testing. These patients had less hypotension, higher blood pressure, and did not require any intravenous fluids during or after the test; patients were discharged earlier than those who did not ingest the hydration solution prior to the test.

The cause-specific mortality of all GHD children and adults in Denmark was reported by Kirstine Stochholm et al. During the years 1980 to 1999, there were 1823 patients with childhood and adult onset GHD; 581 of them died. The mortality rates were higher among all groups of GHD patients, as compared with appropriately matched controls. Furthermore, mortality due to cancer was increased; this was evident even in patients who had no evidence of cancer prior to rhGH treatment. Cardiovascular mortality was also increased.

The reproducibility of the insulin-like growth factor (IGF)-I generation test was assessed in 15 adults by Helena Gleeson and Stephen Shalet. Subjects were given rhGH 7 mg on 2 occasions separated by 4 weeks. The incremental response of IGF-I levels had a reasonable reproducibility and the test was considered to be a valid tool to measure GH responsiveness.

The targeting of IGF-I levels as a means to adjusting rhGH dosages in pediatric patients with short stature was studied by John Germak et al. The investigators assessed the effectiveness of an IGF-I-based dosing algorithm in rhGH therapy in a large group of short children. They concluded that the dosing algorithm was effective and allowed the titration of rhGH to the IGF-I levels. This permitted the treatment with rhGH based on the sensitivity of the individual patient. Patients with GHD demonstrated a greater increase in height and higher IGF-I levels as compared with idiopathic short stature patients.

There were several interesting papers regarding the newly available IGF preparations approved for the treatment of primary IGF-I deficiency. Susan Park et al characterized the structure and heterogeneity of mecasermin (rDNA) as a monomeric polypeptide containing 70 amino acid residues and intramolecular disulfite bridges. Enona Gopinath showed that rhIGF-I injections supplied as a refrigerator-stable aqueous formulation had a long shelf-life (12 months) stored at 2°C–8°C.

In other studies, William Barr et al showed that the administration of a single dose of 0.5 mg/kg of rhIGF-I/rhIGFBP-3 to healthy adults was well-tolerated with no significant adverse events and produced a sustained elevation of serum IGF-I levels. These data supported the effectiveness of once-a-day dosing of this preparation. Additionally, Kenneth Attie et al demonstrated that the free levels of circulating IGF-I were sustained at a physiologic range following the administration of 0.5 mg/kg of rhIGF-I/rhIGFBP-3 given to adult volunteers. These data were in contrast to the substantial increase in free IGF-I levels induced by the administration of isolated rhIGF-I.

In a multicenter clinical trial, Cecilia Camacho-Hubner et al assessed the treatment with once daily-rhIGF-I/rhIGFBP-3 dosages on patients with severe primary IGF-I deficiency. There were 47 children from 13 countries given up to 1 mg/kg/day (low-dose group) or up to 2 mg/kg/day (high-dose group) titrated in accordance with the IGF-I levels. The height velocity in the low-dose group increased from 3.4 cm/yr to 6.4 cm/yr. The high-dose group increased the height velocity from a mean of 2.0 cm/yr to 8.3 cm/yr. The bone age advanced proportionately in both groups. Most patients developed antibodies to rhIGF-I/rhIGFBP-3, but this was not associated with adverse effects or growth attenuation. There were other adverse events that were considered mild, including hypoglycemia, headaches, erythema, and lipohypertrophy. Once-daily treatment with up to 2 mg/kg/day was effective and had an acceptable safety profile.

Patients with severe insulin resistance syndrome were treated with rhIGF-I/rhIGFBP-3 by Fiona Regan et al. The author reported improved glycemic control as well as growth in children with Leprechaunism and concluded that rhIGF-I/rhIGFBP-3 was an effective therapeutic agent.

Polycystic Ovary Syndrome (PCOS) and Metabolic Syndrome

In a large scale population study, Mark Goodarzi et al genotyped 3 variants in the FEM1A gene located in chromosome 19 in a cohort consisting of 287 women with PCOS and 187 healthy controls; all subjects were white. The researchers showed that carriers of the allele of rs8111833 had an increased risk of PCOS (odds ratio 2:1), and suggested that the FEM1A gene modulates the development of PCOS.

Susanne Tan et al showed that PCOS patients had an increased risk of metabolic syndrome and Jennifer Wolfang et al showed that lean, non-obese African-American women had double the prevalence rate of insulin resistance and cardiovascular risk factors

compared with Hispanic and non-Hispanic white female counterparts. Race played an important role independent of BMI.

Vitamin D deficiency was linked to metabolic syndrome by Jose Botella-Carretero et al. They demonstrated that 60% of such patients had low serum 25-hydroxyvitamin D concentrations which may contribute to insulin resistance.

Pubertal Gynecomastia

Two ingredients common in many hair and skin products were linked to abnormal development of breasts in males with pubertal gynecomastia. Derek Henley et al showed that lavender and tea tree oil contained in personal care products turned on estrogen-regulated genes and inhibited an androgen-regulated gene in human breast cancer line MCF-7. Patients' breast size decreased after they stopped using these products.

Thyroid

Mario Salvi et al showed the immunosuppressive drug rituximab was shown to exert a significant positive effect in the treatment of Graves' ophthalmopathy. The monoclonal antibody rituximab blocked the production of B lymphocytes, particularly from the orbital area, thereby modifying the immune response and improving the ophthalmopathy.

Fima Lifshitz, MD

Aromatase Inhibitor Effect on Near-final Height of Boys with Constitutional Delay of Puberty

Hero et al reported near-final height of boys with constitutional delay of puberty (CDP) treated during adolescence with the aromatase inhibitor, letrozole (Lz). Seventeen boys with CDP were randomized to receive testosterone (T) enanthate 1 mg/kg intramuscularly every 4 weeks for 6 months in combination with placebo (Pl; n = 8), or letrozole 2.5 mg/day orally (n = 9) for 12 months. Patients were followed to final height. Boys treated with T + Lz reached a higher mean near-final height than boys treated with T + Pl (175.8 vs 169.1 cm, respectively, $P = 0.04$). Near-final heights of subjects treated with T + Lz did not differ from their mid-parental target height (175.8 vs 177.1 cm, respectively, $P = 0.38$), while near-final heights of T + Pl-treated boys were lower than their mid-parental target height (169.1 vs 173.9 cm, respectively, $P = 0.007$). Patients treated with T + Lz had a greater increment in height SDS than did T + Pl-treated boys (+1.4 vs +0.8 SDS, respectively, $P = <0.03$). The authors concluded that an increase in adult height can be achieved by the use of aromatase inhibitors in adolescent boys with CDP.

Hero M, Wickman S, Dunkel L. Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. Clin Endocrinol. 2006;64:510–513.

Editor's Comment: *Estrogens have been found to be important for bone maturation, growth plate fusion, and cessation of longitudinal growth in both boys and girls. By blocking estrogen biosynthesis in boys with the use of aromatase inhibitors, one could possibly delay bone maturation and improve their final height. Two studies[1,2] have demonstrated an improvement in predicted adult height of 5.1 cm and 5.9 cm following the administration of Lz for one or 2 years to boys with either CDP or idiopathic short stature. This study by Hero et al is the first to report an improvement in the near-final height of boys with CDP treated with T + Lz. The near-final height of subjects treated with Lz did not differ from their mid-parental target height, while the near-final height was found to be lower than the mid-parental target height in boys treated with placebo. It is of interest to note that the delay in bone maturation achieved during treatment with Lz was maintained after cessation of treatment, as indicated by the more delayed bone age at near-final height in the Lz-treated boys. In all 3 of these studies, Lz effectively inhibited estrogen biosynthesis, as indicated by low estradiol and elevated FSH, LH, and testosterone concentrations in the Lz-treated group. Six months after the cessation of treatment, the concentrations of gonadotropins, T, and estradiol did not differ among patients treated with Lz and Pl.*

Larger numbers of patients, particularly short boys with idiopathic short stature and relatively early puberty, need to be studied to confirm these findings. Due to the gonadal androgen secretion noted during aromatase inhibition, careful follow-up of the progression of puberty, maturing spermatogenesis, and high-density lipoproteins of treated patients is necessary. In addition, the effect of low levels of estrogens on bone mass accrual during puberty and on body composition needs to be carefully followed. However, one could envision that this form of therapy could prove to be at least as effective as growth hormone and/or gonadotropin-releasing hormone analogs in increasing the final height of boys with idiopathic short stature entering into puberty at a relatively early age.

Roberto Lanes, MD

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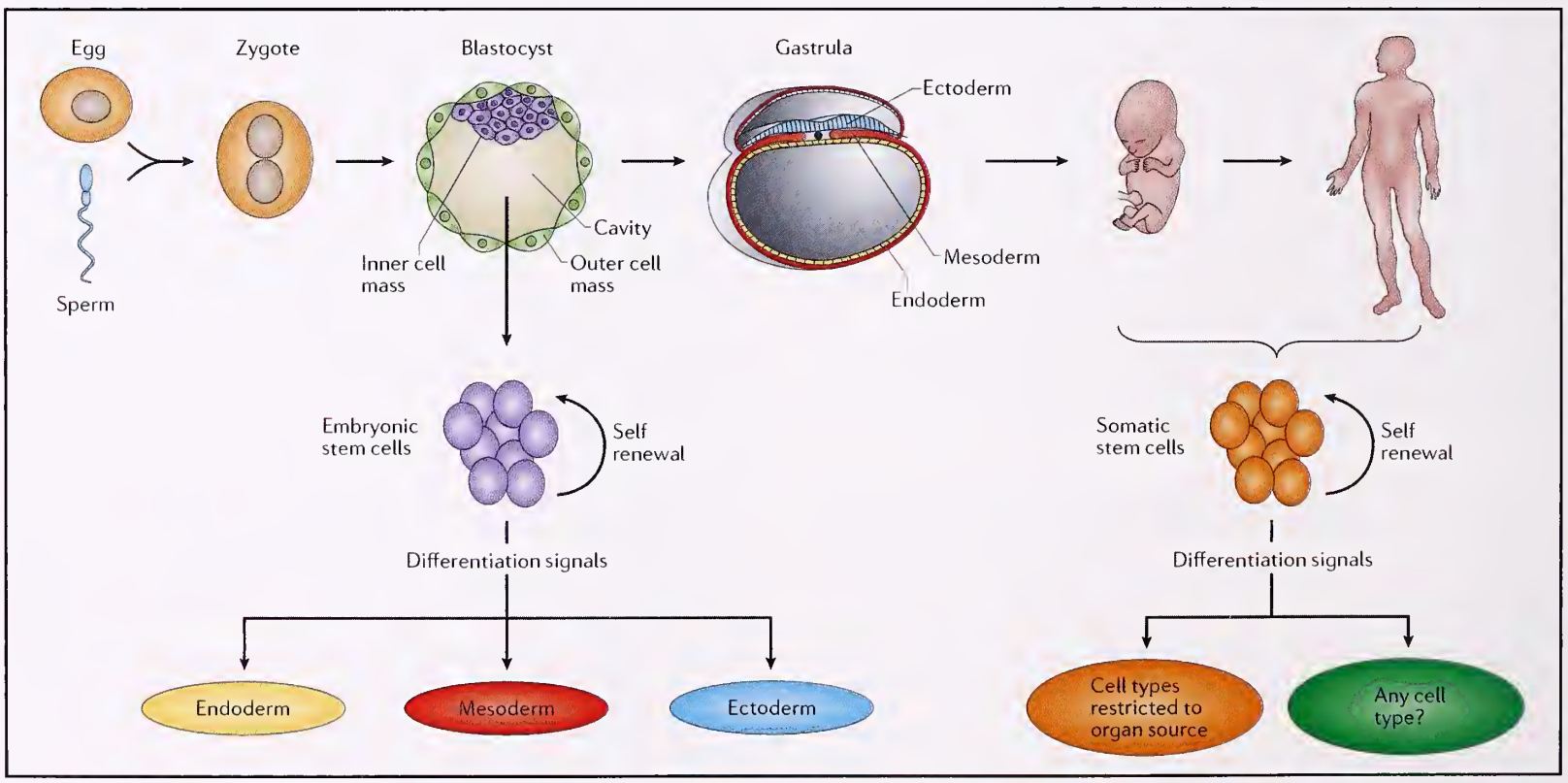
Genetic Medicine: Dream, Reality or Something in Between?

Genetic medicine has had its ups and downs and is often influenced more by rapid shifts in public sentiment than by scientific progress. Accordingly, a thoughtful and objective review of genetic medicine from O'Connor and Crystal is welcome and appreciated by those of us who are not familiar with the intricacies and recent progress in the field. Focusing on treatment of monogenic disorders, it examines the current status of 3 broad categories of genetic medicines: somatic stem cells, gene transfer, and RNA modification.

The review begins with the challenges facing genetic medicine. The main barriers are the delivery and maintenance of new genetic information. For stem-cell therapies, the major issues involve immune surveillance against foreign cells, providing a 'niche' and selective advantage for the transplanted cells and controlling and coordinating the proliferation, differentiation, location and survival of the stem cells and their progeny (Figure). For gene-transfer approaches, success requires circumventing immune defenses that arise against vectors that carry the therapeutic genes, transferring the gene to a sufficient number of cells to modify the mutant phenotype, and controlling expression of the new gene. For RNA-modification therapies, the principal challenge is delivery and, to some extent, specificity. The authors stress that to overcome these challenges, it is essential to understand the target, including the molecular basis of the disorder, its mode of inheritance, the range of mutations and genotype-phenotype relationships that produce disease phenotypes, and how the manifestation of these phenotypes are influenced by age, location in the body, and modulation by other genes.

The review explains and illustrates the different therapeutic approaches and defines many of the terms commonly used in the world of genetic medicine. For example, differences between commonly used viral and non-viral gene-transfer vectors and their advantages and disadvantages are defined, as are the differences between antisense oligonucleotide, RNAi, ribozyme, and *trans*-splicing strategies to therapeutically alter mRNA transcripts harboring disease-causing mutations. Particularly interesting is a compilation and discussion of gene-transfer trials with a cautious assessment of their success in correcting the disease phenotype. The authors seem to be critical of the lack of success in many instances, but also optimistic that much has been learned to provide a basis for future progress.

With regard to future prospects, the authors ask the question: with all the human and financial resources



Embryonic and somatic stem cells as a source of genetic medicines. The fusion of sperm and egg gametes during human fertilization establishes a diploid zygote and initiates a series of cell divisions that result in a multicellular embryo. The blastocyst stage is characterized by the presence of a blastocyst cavity, outer cell mass and inner cell mass. Embryonic stem cells are derived from the inner cell mass of the blastocyst. Embryonic stem cells in culture are capable of self-renewal without differentiation and are able to differentiate into all cell types of the endoderm, mesoderm and ectoderm lineages using appropriate signals. In utero, the blastocyst implants and all three embryonic germ layers are formed during gastrulation. Somatic stem cells are present in many fetal and post-natal tissues. Somatic stem cells are also capable of self-renewal and, with appropriate signals, differentiate into various cell types from the organ from which they are derived. The extent to which they are capable of differentiating into cell types from alternative lineages is controversial.

that have focused on using genetic medicines to treat monogenetic disorders, why don't any of the therapies that have been tried alter disease phenotypes in a reproducible, efficacious manner, without significant toxicity? Their answer is that drug development takes years, averaging 12 to 15 years from concept to government approval. They also point to large societal hurdles that result in regulatory delays as well as economic barriers that must be overcome. They emphasize that despite its attention, the genetic medicine field is still young and that while genetic medicine is simple in concept, it is challenging to make it a reality. Indeed, they underscore the fact that paths for development of ground-breaking therapies taken as standard today, such as bone marrow and internal organ transplantation, and *in vitro* fertilization, were littered by disappointments and nay-sayers who predicted inevitable failure.

O'Connor TP, Crystal RG. Genetic medicines: treatment strategies for hereditary disorders. Nat Rev Genet. 2006;7:261–276.

Editor's Comment: *This is an excellent review for clinicians and other readers of GGH who want to catch up on the current status of genetic medicine. In most ways, the potential use of genetic medicine to treat problems of skeletal growth faces the same problems as mentioned for other areas. For instance, chondrocytes within the avascular growth plate represent a very difficult cell to target by any of the strategies mentioned in this review, as well as by more conventional therapies. There are social hurdles to developing growth-stimulating therapies in some segments of our culture, and there are enough differences between patients and animal models to make testing of new therapeutic approaches in animals challenging. Nevertheless, it seems highly likely that the general advances in genetic medicine predicted by the authors of this review will find their way to more effective ways to treat growth problems, especially in monogenetic disorders.*

William A. Horton, MD

Catch-down of SGA and AGA Infants Born to Short-statured Parents

Völkl and colleagues reported their findings of a cross-sectional analysis of linear growth during the first 4 years of life. The 96 subjects (38 females) were children, between 5 and 10 years of age, who were born to short parents and presented to the pediatric endocrine clinic for evaluation of short stature. Endocrine disorders were excluded in each case. All the children had familial short stature (FSS) defined as a height SDS ≤–2.0 but within the normal range of parental height according to the calculated target height. At least one of the parents had short stature (height ≤–2 SDS). Children were divided into 2 groups according to birth size; 41 (19 female) were in the small for gestational age (SGA) group for whom birth length or birth weight was ≤–2.0 SDS, and 55 (19 female) were in the group of appropriate for gestational age (AGA) infants. All children were born at >36 weeks gestation and none were receiving any chronic medications. Cross-sectional data for length/height/weight/head circumference for the first 4 years of life were collected retrospectively from standardized German growth charts. The data analyses were performed at birth, 1, 2, and 4 years of age. The SDS of height and height velocity were calculated according to German and Swedish reference data.

There was a significant ($p<0.0001$) decline of height SDS within the first 2 years of life, which was more prevalent in the AGA children. These children started with a mean height of 0.09 SDS ± 1.02 SDS at birth and ended up with a mean height of –2.36 ± 0.72 SDS at 4 years of age. The growth pattern of the SGA group was similar, but the height loss was less than for the AGA group (mean –2.04 SDS at birth, –3.05 SDS at 4 years). Even though the height of the SDS did not decrease as much as that of the AGA children, there was a significant difference between the mean height SDS data at all of the times studied. The absolute difference between height SDS values narrowed during the observation period. There was no significant difference in height and BMI SDS between those children having a father with short stature, compared with those with a mother with short stature. In addition, there was no relationship between the child's gender and the gender of the short parent.

The authors pointed out that there was selection bias inherent in their study since all children were initially identified in the pediatric endocrine clinic where they had been referred for evaluation of short stature. In addition, the short-stature children born SGA belonged to a subgroup of SGA children who did not experience postnatal catch-up growth. The authors stated that there is minimal information in the literature of spontaneous growth during the first years of life in children with idiopathic short stature born AGA. Of note, the SGA children increased their BMI to the same level as the AGA group after one year of age, but then these children tended to have a lower BMI SDS during the following years. This result was consistent with population-based data showing that SGA children weigh significantly less than AGA children at 3 to 6 years of age. The etiology of the growth failure in these children remains undefined.

Völkl TM, Haas B, Beier C, Simm D. Dorr HG. Catch-down growth during infancy of children born small (SGA) or appropriate (AGA) for gestational age with short-statured parents. J Pediatr. 2006;148:747–752.

First Editor's Comment: *Völkl and his colleagues have provided some very interesting data with regard to growth patterns in children identified as having familial short stature at 4 years of age. Both AGA and SGA children have losses in SDS over the first 4 years of life, but the loss appears to be greater in those children born SGA. The change in*

SDS is greater for those who were born AGA. As noted by the authors, the study was retrospective and it would be important to perform prospective studies on the children born SGA. Performance of these studies on the AGA children with short parents might prove more difficult, but the information to be gathered from such a study might be extremely important in understanding the auxilogical changes that occur in these children, and might lend support to therapies for improving final adult height.

William L. Clarke, MD

Second Editor's Comment: *The body weight and growth progression of patients with FSS with or without constitutional growth delay (CGD) was studied by Dr. Vaquero-Solans and me.[1] The linear growth in infancy was similar in both groups of patients. Infants with FSS and CGD showed a sharp parallel fall from the 50th percentile to –1 SD by 3 months of age and a more gradual, but steady, deterioration in length to –2 SD between 3 to 27 months of age. The z scores of height for age remained 2.0 – 2.5 SD below the mean until 12 years of age. In contrast, the body weight progression differed among the 2 types of patients. The CGD patients exhibited a marked impairment in body weight gain as compared with the FSS. Patients with CGD had body weight deficits for stature, whereas the FSS patients did not. The differences were more marked during infancy. The CGD patients attained an appropriate body weight for height by 9 to 10 years of age, whereas the FSS patients presented body weight excess after 4 years of age and remained progressively overweight until 12 years of age. The catch-down pattern of growth in CGD patients during infancy has been observed by others.[2] The growth data of SGA and AGA infants in the paper by Völkl et al was similar to the growth exhibited by FSS patients, though they did not assess bone development or weight and height progression after 4 years of age. The pattern of growth and weight gain during infancy and childhood has become more important as it may set the stage for obesity and adult-onset disease.[3,4]*

Fima Lifshitz, MD

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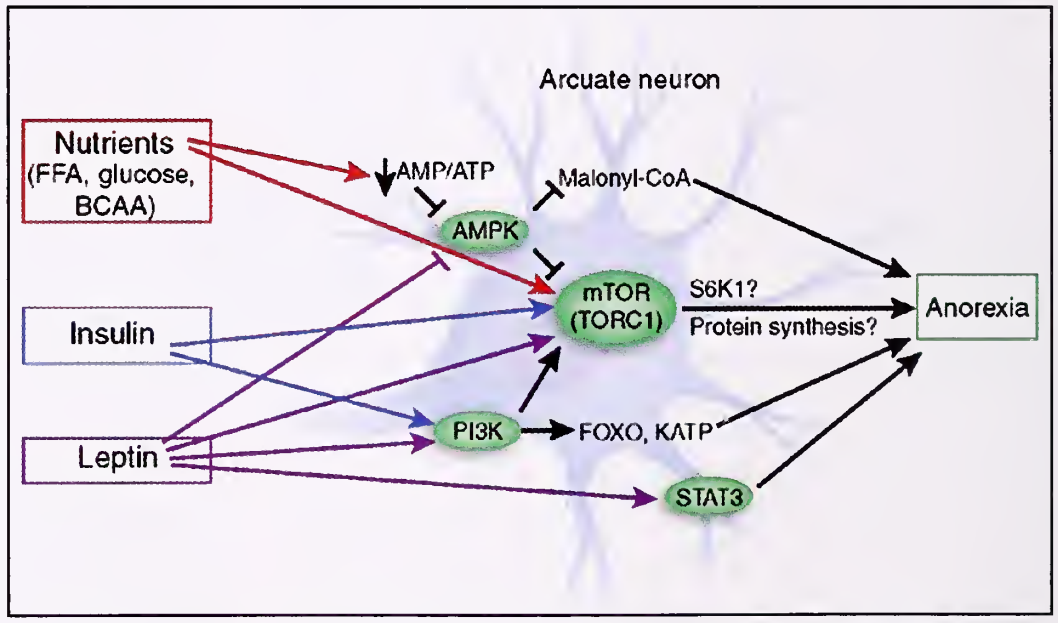
Interleukin Deficiency Leads To Hyperphagia, Obesity, and Insulin Resistance

Serum concentrations of interleukin 18 ([IL-18] OMIM 600953, chromosome 11q22.2-q22.3), an interferon-γ inducing factor that augments natural killer cell activity and perhaps contributes to chronic inflammatory disorders such as Crohn's disease, are increased in patients with obesity, type 2 diabetes mellitus, and polycystic ovarian syndrome. Interleukin-18 is synthesized and secreted by hepatic Kuppfer cells and macrophages. The biologic effects of IL-18 are mediated by its binding to a specific cytokine receptor (IL-18R1; OMIM 604494, chromosome 2q12) and receptor accessory protein (IL-18RAP; OMIM 604509, chromosome 2q12). The biologic activity of IL-18 is inhibited by binding to an IL-18-binding protein ([IL-18BP] OMIM 604113, chromosome 11q13) which prevents the interaction of IL-18 with IL-18R1.

Netea et al demonstrated in the mouse that loss ("knock out") of IL-18 (*IL-18*$^{-/-}$), or its receptor (*IL-18r*$^{-/-}$), or excessive ("knock in") production of *IL-18bp* (thus neutralizing endogenous IL-18) results in hyperphagia and obesity associated with hyperinsulinemia and insulin resistance primarily confined to muscle



Converting metabolic signals into anorectic (appetite-suppressing) responses in the hypothalamus. Major classes of anorectic signals in the hypothalamus include nutrients such as free fatty acids (FFA), glucose, leucine and other branched-chain amino acids (BCAA), and hormones such as insulin and leptin. Cota et al[1] show that BCAA potently activates signaling through the mTOR complex (TORC)-1. FFA and glucose may also regulate TORC1 in the arcuate nucleus, either directly or indirectly (via cellular AMP/ATP levels and AMPK activity). The regulation of cellular malonyl–coenzyme A levels may mediate a component of feeding control by AMPK in parallel with AMPK effects on mTOR. In addition to potentially regulating TORC1 indirectly through the inhibition of AMPK, insulin and leptin may also control mTOR via the PI3K or other pathways. Regulation of FOXO-dependent transcription and ATPdependent potassium (KATP) channels probably also contributes to PI3K-dependent anorexia. Activation of STAT3-dependent transcription by leptin is a crucial short- and long-term regulator of feeding. Although the mediators of TORC1-dependent anorexia are not clear, S6K1 and downstream events such as protein synthesis are likely to be involved.

and adipose tissues, hyperglucagonemia, hyperglycemia and impaired glucose tolerance, increased hepatic glucose output, hyperlipidemia, and vascular atherosclerosis. Thus, *IL-18*$^{-/-}$ mice had characteristics of metabolic syndrome. In *IL-18*$^{-/-}$ mice, relative to wild-type (wt) mice, body weight was normal at 3 months of age but substantially elevated by 6 months, and became progressively greater thereafter. The increased weight of the *IL-18*$^{-/-}$ mouse was due to excessive caloric intake and augmented fat accumulation, while basal metabolic rate remained normal. Peripheral administration of leptin and central injection of recombinant IL-18 decreased appetite; peripheral administration of IL-18 restored glucose homeostasis in the *IL-18*$^{-/-}$ mouse. The increase in hepatic glucose production in the *IL-18*$^{-/-}$ mouse was due to decreased phosphorylation of the transcription factor—signal transducing and activation of transcription (STAT)3—that resulted in accentuated gluconeogenesis due in part to increased expression of phosphoenolpyruvate carboxykinase (PEPCK-1). The investigators concluded that IL-18 is another component of the complex of factors that regulate appetite and energy metabolism.

Netea MG, Joosten LA, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nature Med. 2006;12:650–656.

Editor's Comment: *To the enlarging list of anorexigenic factors (insulin, leptin, α-MSH, cocaine and amphetamine regulated transcript [CART], branched chain amino acids, and other nutrients) that regulate appetite and energy expenditure, IL-18 may now be added. One could speculate that an analogue of this cytokine might be an effective therapeutic agent for the management of patients with obesity and/or metabolic syndrome. Recent studies have further defined cellular mechanisms involved in appetite regulation. The serine-threonine kinase mTOR (mammalian target of rapamycin) has been identified as a critical regulatory factor in the integration of peripheral hormonal and nutritional (glucose, fatty acids, amino acids) signals (Figure) that decrease appetite.*[1,2] *Leptin, insulin, and various nutrients suppress appetite in part by activating mTOR. This protein is a component of the multi-protein complex TORC1 that senses energy availability; when energy is sufficient, TORC1 permits cell growth and enables leptin production by the white fat cell. The TORC1 is particularly active in the arcuate nucleus, the site in which the central regulation of energy balance is present. Leptin also decreases appetite and energy utilization by inhibiting synthesis of orexigenic agouti-related peptide (Agrp) in the arcuate nucleus, an activity mediated through phosphatidylinositol 3 kinase (PI3K) but antagonized by the forkhead box-containing protein of the O subfamily (FOXO1), a DNA binding protein.*[3]

Allen W. Root, MD

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Insulin and Sulfonylureas in Diabetic Patients with Kir6.2 Mutations

Neonatal diabetes mellitus is a rare disorder and about half of those diagnosed before 6 months of age develop permanent diabetes. The most frequently identified genetic cause is related to heterozygous activating mutations in the *KCNJ11* gene encoding the Kir6.2, a subunit of the ATP-sensitive potassium (K_{ATP}) channel. The activity of this channel in the pancreatic beta cell regulates insulin secretion. These activating mutations cause 30% to 58% of the cases of diabetes mellitus diagnosed in infants. Diabetes results from a failure of this channel to close in response to increased intracellular ATP, leading to impaired insulin secretion. Sulfonylureas, a class of drugs used to treat type 2 diabetes mellitus, close this potassium channel by an ATP-independent route, causing insulin secretion. Thus, this drug represents an alternative therapy to insulin in these patients. The first cases treated with sulfonylureas were reported 2 years ago; this study by Pearson et al is the first to assess the sustained response to sulfonylureas in a large cohort of patients who were initially treated with insulin.

A total of 49 consecutive patients who had been diagnosed at less than 6 months of age with Kir6.2 mutations were switched from insulin to sulfonylurea therapy. An adequate dose of sulfonylureas was defined as a dose of glyburide (also known as glibenclamide) of at least 0.8 mg/kg/day. The change was considered to be successful if the patient was able to stop insulin treatment completely. Additionally, insulin secretory responses were assessed in subgroups receiving intravenous or oral glucose, a mixed meal, or intravenous glucagon before and after treatment with glyburide.

Switching was successfully accomplished in 44 patients regardless of the type of sulfonylurea used, suggesting a class effect. The oldest patient was 36 years of age and the youngest was 3 months of age. The mean glycated hemoglobin level improved in all subjects and fell from 8.1% during insulin therapy to 6.4% after a mean of 12 weeks of sulfonylurea treatment and cessation of insulin. The initial improvement was sustained in the 12 patients who were insulin-independent for more than one year. The longest duration of insulin independence was 2.0 years, with a glycated hemoglobin level of 5.7%.

Switching to sulfonylureas was unsuccessful in only 5 patients (10%). Of these patients, 4 (80%) had severe neurological features, including severe developmental delay, epilepsy, and neonatal diabetes, known as DEND syndrome. These neurological features occurred in only 6 patients (14%) who were successfully treated with

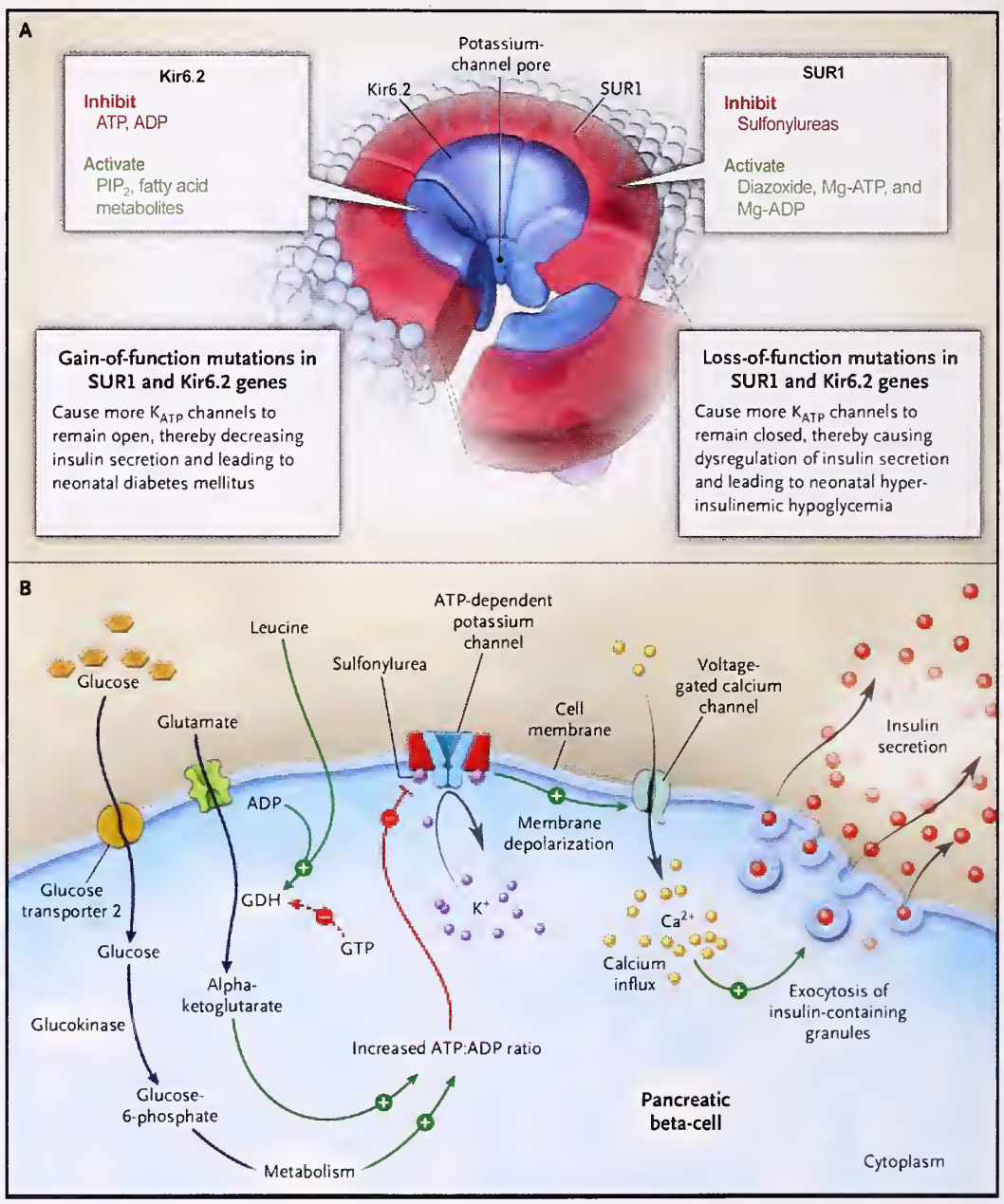
sulfonylureas. In 2 families, the mothers were unable to switch from insulin therapy, even though their affected children were able to do so. Only 5 patients had transitory diarrhea while on sulfonylureas. The treatment had no detrimental effect on growth. There were no patient reports of severe hypoglycemia.

In physiological studies sulfonylurea treatment increased insulin secretion. This was more highly stimulated by oral glucose or a mixed meal than by intravenous glucose. Exogenous glucagon increased insulin secretion only in the presence of sulfonylureas.

The successful switch from insulin to sulfonylureas was reflected *in vitro* in xenopus oocytes with the same K_{ATP} channel mutation. Tolbutamide blocked more than 75% of the K_{ATP} current. The relatively high doses of sulfonylureas used in the treatment of these patients appeared to be safe on the short term. There was no increase in mild-to-moderate hypoglycemia, and a near-to-normal glycated hemoglobin level was achieved. The improved insulin secretory response to oral glucose and to mixed meals was interpreted as an effect of the sulfonylureas on the K channel, allowing the membrane to become depolarized, thereby the beta cell was able to respond to endogenous incretins (glucagon-like peptides). Because of the reported important therapeutic implications, the authors recommended a molecular diagnosis in all patients with neonatal diabetes mellitus diagnosed before the age of 6 months. It is also stressed that a longer follow-up is required to fully appreciate this progress in therapy of a genetic form of diabetes.

Pearson ER, Flechtner I, Njølstad PR, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. N Engl J Med. 2006;355:467–477.

Editor's Comment: *This large collaborative study by the neonatal diabetes international collaborative group has confirmed and extended our knowledge of the treatment of the most frequently occurring genetic form of permanent neonatal diabetes resulting from activating Kir6.2 mutations of an ATP-sensitive potassium channel of the beta cell (Figure). This novel pharmacogenetic approach was based on a model of regulation of insulin secretion involving a K_{ATP} channel. Interestingly, inactivating mutations of the components of this channel have been identified as the cause of hyperinsulinemic hypoglycemia of infancy; an opposite condition, activating mutations causing diabetes mellitus by limiting insulin secretion. The authors not only carefully investigated the new treatment with sulfonylureas, but also established physiologic evidence that this treatment restored insulin secretion in*



Regulation of insulin secretion. The Kir6.2–SUR1 complex and its regulation and genetic variability. Panel A shows the detailed subunit structure of the K_{ATP} channel. Panel B shows the regulation of insulin secretion by glucose or amino acids (glutamate is used in this example). The beta cell senses the concentration of glucose or amino acid, or both, and converts their metabolism to energy in the form of ATP. In turn, ATP is converted to changes in the electrical membrane that regulate voltage-gated calcium channels to permit the influx of calcium and thereby insulin secretion. Central to these processes is the K_{ATP} channel, which is composed of four small subunits, Kir6.2, that surround a central pore and four larger regulatory subunits constituting SUR1. In the normal resting state, the potassium channel is open, modulated by the ratio of ATP to ADP. Hence, the beta-cell membrane is hyperpolarized, and the voltage-gated calcium channel (L type) remains closed. With the ingestion of food, the glucose concentration rises and enters the beta cell by way of the non–insulin-dependent glucose transporter 2. Glucose is rapidly phosphorylated by glucokinase, yielding glucose-6-phosphate, and further metabolism yields energy-rich ATP. The now altered ratio of ATP to ADP closes the K_{ATP} channel, causing the accumulation of some intracellular potassium, membrane depolarization, opening of the voltage-regulated calcium channel, and triggering of insulin exocytosis. PIP2 denotes phosphatidylinositol-4,5-bisphosphate.

relation to glucose metabolism by closure of mutant K_{ATP} channels; it also amplified the effect of incretins levels that are stimulated by nutrient ingestion.

In an accompanying editorial, Sperling[1] recommended that a test for this genetic mutation be included as part of routine newborn screening programs. In all cases, newborns with this disease should be tested for activating mutations affecting Kir6.2, an approach facilitated by the one exon structure of the gene. Furthermore extensive familial studies are needed and other phenotypes may be expected as a consequence of mutations with milder activity. Another cause of permanent neonatal diabetes was also reported by Babenko et al.[2] A careful history is needed in all patients with the onset of diabetes in infancy. It is remarkable that some, but not all, adult patients were responsive to the treatment switch from insulin to sulfonylureas. More information is needed regarding the failures observed in about 10% of the patients with the same genetic mutations.

Raphaël Rappaport, MD

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Acidosis and Protein Kinase: A Novel Mechanism of Growth Failure

Chronic acidosis is known to cause growth failure by an effect on the bone end-organ, but the exact mechanism has remained elusive. Goldberg and colleagues have recreated growth retardation of endochondrial ossification centers *ex vivo* by culturing murine mandibular condyles in medium with 2.4 mM HCl, to lower the pH to 7.1 – 7.15. In previous studies, this group found that acidosis led to decreased expression of both insulin-like growth factor (IGF)-I and its receptor (IGF-IR) as well as markers of differentiation like type II collagen and cartilage proteoglycans. They also found that the acidotic growth inhibition could be prevented by local application of low concentrations (10^{-10} M) of parathyroid hormone (PTH).

PTH works through 2 main signaling pathways: Gq protein/protein kinase C (PKC) and Gs protein/adenylate cyclase/protein kinase A (PKA) pathway. Goldberg and colleagues previously showed that acidosis represses PKC expression, an effect partially inhibited by PTH. However, the PKC agonist PMA succeeded in protecting condyles against acidotic differentiation arrest (increased expression of type II collagen and proteoglycans) but not the acidotic suppression of IGF-I and IGF-IR expression.[1] Therefore, the researchers sought to examine the possible role of the PKA pathway in acidosis-induced growth retardation.

In contrast to the reduction in PKC levels, PKAα protein levels were increased by acidosis; both levels were normalized by adding PTH to the acidotic cultures. A specific PKA inhibitor, H89, prevented the acidosis-induced reductions in expression of IGF-I, IGF-IR, and aggrecan (the core protein of cartilage-specific proteoglycans in chondrocytes). Using the converse approach, the cAMP regulating factors 8Br-cAMP (a cAMP analog), iso-butyl methyl xanthine ([IBMX] a phosphodiesterase inhibitor), and forskolin (an adenylate cyclase analog), all reproduced the morphologic changes seen in acidotic growth plates: decreased condylar length with loss of the chondroblast population, leaving the mature hypertrophic cell layer adjacent to the chondroprogenitor zone which is itself wider due to differentiation arrest. Chondrocyte proliferation was also reduced by acidosis, IBMX, and forskolin, as evidenced by decreased expression of proliferating cell nuclear antigen (PCNA), a cell cycle marker. Acidosis and IBMX also decreased expression of IGF-IR in the chondroblasts and chondrocytes. Using mandibular condyle-derived primary cultures of chondrocyte (MCDC) cells, the temporal cascade of endochondrial ossification was reproduced. When grown in acidotic conditions for one week, MCDC cells showed less proliferation and developed fewer cartilaginous nodules. The possibility of toxic effects of acidosis acidifying the intracellular cytoplasm was neatly ruled out by comparing the fluorescence pattern of a pH-dependent fluorescent dye, acridine orange; intracellular pH looked normal despite acidotic culture conditions, but reflected intracellular acidosis with the addition of nigericin (an ionophore that equalizes intracellular and extracellular proton concentrations). Involvement of the entire PKA pathway by acidosis was demonstrated by the increased ratio of phosphorylated-(activated) to total cAMP-responsive element binding protein ([CREB] the transcription factor which is a major PKA substrate) at one end, and the increased expression of Gsα protein at the other. Despite acidosis, the Gs inhibitor GDPβS allowed normal condyle development.

Thus, the authors developed a model of growth plate chondrocytes whereby acidosis induces Gsα protein, which in turn activates adenlyate cyclase and hence PKA, leading to phosphorylated CREB and altered gene expression. Both genes of differentiation and IGF-IR are ultimately down-regulated. PTH inhibits the acidotic growth retardation through both its PKA and PKC signaling pathways. The authors speculated that the activation of Gs protein by acidosis was mediated through proton-sensing receptors rather than ligand binding.

Goldberg R, Reshef-Bankai E, Coleman R, Green J, Maor G. Chronic acidosis-induced growth retardation is mediated by proton-induced expression of Gs protein. J Bone Miner Res. 2006; 21:703–713.

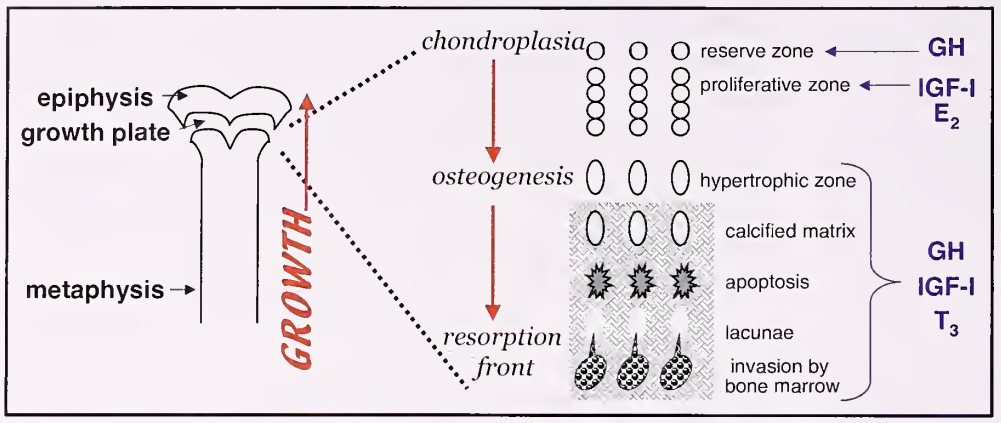

Figure 1. Growth plate and process of growth. Reprinted with permission from: Grimberg A, De Leon D. Disorders of Growth. In Moshang T, Ed., Requisites in Pediatrics - Pediatric Endocrinology, Elsevier, Inc., Philadelphia, 2005; 127–167. Copyright © Elsevier. 2005. All rights reserved.

Editor's Comment: *I am always delighted when the underlying mechanisms of long-standing clinical observations are finally elucidated. To remind our readers, the anatomy of the growth plate and the growth process are depicted in the schematic[2] of Figure 1. The chondroblasts absent in the condyle cultures with acidosis or cAMP regulating factors in the current paper (Figure 2) correspond to the proliferative zone in Figure 1. This zone is regulated primarily by IGF-I and estradiol. Instead, the hypertrophic zone was seen adjacent to chondroprogenitors (Figure 2) in an expanded reserve zone (Figure 1) that failed to fully differentiate.*

Although showing a role of PKA in acidosis-induced growth retardation in the growth plate is certainly novel, this is not the first paper to demonstrate regulation of the IGF axis by PKA. cAMP/PKA induced IGF-I expression in primary rat osteoblasts[3] and cultured embryonic mouse mandibular mesenchymal cells,[4] IGF binding protein (IGFBP)-1 in hepatocytes,[5] and IGFBP-3, -4 and -5 in periosteal and osteoblast bone cell cultures.[6] PKA inhibitors interfered with the induction of IGF-I and IGFBP-3 by growth hormone in porcine ovarian granulosa cells,[7] IGFBP-3 by FSH also in porcine ovarian granulosa cells,[8] and IGFBP-4 by platelet-derived growth factor-BB in fetal rat lung fibroblasts.[9]

Adda Grimberg, MD

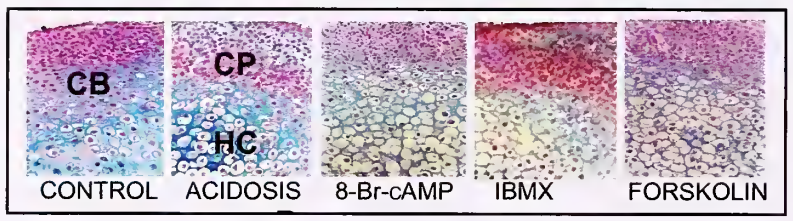

Figure 2. Effects of 8Br-cAMP, IBMX, and forskolin (cAMPrf) on the development of the mandibular condyle. Condyles derived from 6-day-old ICR mice were cultured for 72 h under normal, acidic condition (2.4 mM HCl) or treated with the following cAMP-inducing factors (cAMPrf): 0.05 mM 8BrcAMP (a cAMP analog), 0.05 mM IBMX (a phosphodiesterase inhibitor), or 1 μM forskolin (anadenylyl cyclase analog). Chondroblastic cell layer (CB) is absent in the acidosis and cAMPrf cultures, leaving the hypertrophic cells (HC) adjacent to the chondroperichondrium zone (CP), which is larger than that of the control.
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